8

Extrapolation of Animal Toxicological Data to Humans

8.1 Introduction

A full evaluation of the health effects of ozone (O_3) requires an integrated interpretation of human clinical, epidemiological, and animal toxicological studies. Each of these three research approaches has inherent strengths and limitations. Animal toxicological data are valuable because they provide concentration- and duration-response information on a fuller array of effects and exposures than can be studied in humans. However, historically, use of animal toxicological data has been limited because of difficulties in quantitative extrapolation to humans. Recent advances in the state-of-the-art of extrapolation have reduced several uncertainties, which will be discussed in this chapter.

Qualitative animal-to-human extrapolation generally is accepted because O_3 causes similar types of effects in several animal species, from mouse to nonhuman primate (Chapter 6). Also, when similar endpoints (e.g., inflammation and pulmonary function) have been examined in O_3-exposed animals and humans, similar effects are observed. However, quantitative extrapolation (i.e., if a certain exposure causes a specific effect in animals, what exposure is likely to cause that same effect in humans?) is the goal but is controversial. Such an extrapolation requires an integration of dosimetry and species sensitivity. *Dosimetry* is defined as the dose delivered to a site in the respiratory tract (RT). As can be seen in Section 8.2, substantial information is available on dosimetry in several species, including humans. Dosimetric studies that are referenced in the earlier O_3 criteria document (U.S. Environmental Protection Agency, 1986) are summarized only briefly here; newer research is the focus. Species sensitivity, discussed in Section 8.3, refers to the sensitivity of a specific species to the delivered dose. For example, even if the same dose of O_3 were delivered to a specific respiratory tract site in rats and humans, differences in species sensitivity to that dose are likely because of variations in defense mechanisms and perhaps other factors. Section 8.3 also provides a more holistic approach to extrapolation by quantitatively comparing exposure-response data obtained in animals and humans. Sections 8.4 and 8.5 are intended to draw the forgoing information together, reaching conclusions about the potential for acute and chronic human health effects based on animal studies. Lastly, Section 8.6 presents the summary and major conclusions from the chapter.

Although this chapter focuses on animal-to-human extrapolation, dosimetric studies also can be used to elucidate interpretations of the human studies described in Chapter 7. For

example, knowledge of dosimetry in humans as related to age and exercise can enhance understanding of human susceptibility factors.

8.2 Ozone Dosimetry

8.2.1 Introduction

Dosimetry refers to measuring or estimating the quantity or rate of a chemical absorbed by target sites within the RT. The compound most directly responsible for toxic effects may be the inhaled gas O_3 or its chemical reaction products. Complete identification of the actual toxic agents and their integration into dosimetry are complex issues that have not been resolved. Thus, most dosimetry investigations are concerned with the dose of the primary inhaled chemical. In this context, a further confounding aspect can be the units of dose (e.g., mass retained per breath, mass retained per breath per body weight, mass retained per breath per respiratory tract surface area). That is, when comparing dose between species, what is the relevant measure of dose? This question has not been answered; units are often dictated by the type of experiment or by a choice made by the investigators.

Experimental and theoretical (dosimetry modeling) studies are used to obtain information on dose. Experiments have been carried out to obtain direct measurements of absorbed O_3 in the RT, the upper RT (URT; region proximal to the tracheal entrance), and the lower RT (LRT; region distal to tracheal entrance); however, experimentally obtaining dosimetry data is extremely difficult in smaller regions or locations, such as specific airways or the centriacinar region (CAR; junction of conducting airways and gas exchange region), where lesions caused by O_3 occur (see Chapter 6, Section 6.2.4). Nevertheless, experimentation is important for determining dose, making dose comparisons between subpopulations and between different species, assessing hypotheses and concepts, and validating mathematical models that can be used to predict dose at specific respiratory tract sites and under more general conditions.

Theoretical studies are based on the use of mathematical models developed for the purposes of simulating the uptake and distribution of absorbed gases in the tissues and fluids of the RT. Because the factors affecting the transport and absorption of gases are applicable to all mammals, a model that uses appropriate species or disease-specific anatomical and ventilatory parameters can be used to describe absorption in the species and in different-sized, aged, or diseased members of the same species. Importantly, models also may be used to make interspecies and intraspecies dose comparisons, to compare and reconcile data from different experiments, to predict dose in conditions not possible or feasible experimentally, and to better understand the processes involved in toxicity.

8.2.2 Summary of 1986 Review of Experimental and Theoretical Dosimetry

A summary of the more relevant experimental and theoretical dosimetry studies contained in the previous O_3 criteria document (U.S. Environmental Protection Agency, 1986) is presented. The reader is referred to the earlier document for completeness.

Experiments on the nasopharyngeal removal of O_3 in laboratory animals suggested that the fraction of O_3 uptake depends inversely on flow rate (Yokoyoma and Frank, 1972), uptake was greater for nose than mouth breathing (Yokoyoma and Frank, 1972), and tracheal and chamber concentrations were related linearly (Yokoyoma and Frank, 1972; Miller et al., 1979). Only one investigation measured uptake by the LRT, finding 80 to 87% uptake by the LRT of dogs (Yokoyoma and Frank, 1972). At the time, there were no reported results for

human URT or LRT uptake. With the exception of two relatively crude studies by Clamann and Bancroft (1959) and Hallett (1965), there were no data on O_3 uptake in humans at the time of the earlier criteria document (U.S. Environmental Protection Agency, 1986).

Several mathematical dosimetry models were developed to simulate the processes involved in O_3 uptake and to predict O_3 uptake by various regions and sites within the RT. The model of Aharonson et al. (1974) was used to analyze nasopharyngeal uptake data. Applied to O_3 data, the model indicated that the average mass transfer coefficient of this region and the mass retained increased with increasing air flow, but the percent uptake decreased.

Models were developed to simulate LRT uptake (Miller et al., 1978, 1985). The models were very similar in their treatment of O_3 in the airways and airspaces and in their use of morphometric data to define the dimensions of the air compartments and liquid lining. Both the 1978 and the 1985 models of Miller and co-workers took into account reactions of O_3 with constituents of the liquid lining. However, these models differed in their treatment of chemical reactions in the liquid lining, and the later model included transport and chemical reactions within tissue and blood, whereas the first model did not (an instantaneous reaction at the liquid-tissue interface was assumed, so the O_3 concentration was defined as zero). In both models, tissue dose was defined as the O_3 flux to the liquid-tissue interface. Both models predicted O_3 tissue dose to be relatively low in the trachea, to increase to a maximum in or near the CAR, and then to decrease distally; this was characteristic for both the animal and the human simulations (Miller et al., 1978, 1985).

Prior to 1986, there were no experimental results that were useful in judging the validity of the modeling efforts. However, a comparison of the results of Miller and co-workers with morphological data (showing the CAR to be most affected by O_3; see Chapter 6, Section 6.2.4) indicated qualitative agreement between the site of predicted maximum tissue dose and the site of observed maximum morphological damage in the pulmonary region.

8.2.3 Experimental Ozone Dosimetry Data

8.2.3.1 Introduction

Models of O_3 uptake in the RT have reached a scale of sophistication that provide some highly specific predictions regarding the location and magnitude of O_3 dose. However, before these models can be exploited to their fullest degree in extrapolating dose within and between species, validation of the models with experimental data is essential. This section will review the experimental database on which the modeling of O_3 dosimetry is both based and validated. This will help facilitate discussion of the models themselves in subsequent sections. Table 8-1 provides a summary of all post-1986 experimental O_3 dosimetry studies.

8.2.3.2 In Vivo Ozone Dosimetry Studies

The model predictions of Overton et al. (1987), based on the original model of Miller et al. (1985), provided specific predictions about the regional and total uptake efficiencies of O_3 in laboratory rats. It was therefore necessary to test these predictions with actual data. The first data on total RT uptake of O_3 in rats were obtained by Wiester et al. (1987). Ozone uptake was measured in 30 awake, unanesthetized Sprague-Dawley (S-D) rats receiving a nose-only exposure. Each rat was situated within a plethysmograph that continuously monitored the animal's breathing pattern. Air with O_3 flowed by rats' noses at 1,200 mL/min for 1 h at a concentration of 0.3, 0.6, or 1.0 ppm O_3. Determination of RT

Table 8-1. Experimental Studies on Ozone Dosimetry[a]

Type of Study	Species (Strain)	Uptake	Breathing Patterns	Results	Reference
In vivo, nose only	Rat (S-D)	Total RT	V_T = 2.8 mL f = 150 bpm $\dot{V}_E$ = 400 mL/min	Uptake measured in 30 rats exposed to 0.3, 0.6, or 1.0 ppm O_3 for 1-h. Uptake measured using mass balance. Total RT uptake efficiency measured at 40%. Uptake efficiency was independent of O_3 concentration.	Wiester et al. (1987)
In vivo	Rat (F344) Rat (S-D) Rat (Long-Evans) Guinea pig (Hartley)	Total RT	For rats: V_T = 2.6 mL f = 120 bpm $\dot{V}_E$ = 330 mL/min For guinea pigs: V_T = 2.4 mL f = 77 bpm $\dot{V}_E$ = 188 mL/min	All uptake measurements at 0.3 ppm O_3. In addition F344 rats were measured at 0.6 ppm. Uptake measurements made with system of Wiester et al. (1987). Total RT uptake efficiency averaged 47% and was strain and species independent. Uptake efficiency again was shown to be independent of O_3 concentration in F344 rats.	Wiester et al. (1988)
In vivo	Rat (F344)	Total RT, head, larynx/trachea, lung	V_T = 2.05 mL f = 150 bpm $\dot{V}_E$ = 290 mL/min	Regional uptake measured by assaying for recoverable ^{18}O from respiratory tract tissue after animal inhaled ^{18}O-enriched 1 ppm O_3 for 2 h. Fifty-four percent of inspired O_3 was taken up by total RT. Of the O_3 taken up, 49.6% taken up by the head, 6.7% by the larynx/trachea, and 43.6% by the lungs.	Hatch et al. (1989)
In vivo	Human	Total RT, URT, LRT	V_T = 800 mL f = 12 and 24 bpm $\dot{V}_I$ = 350 and 634 mL/s oral, nasal, and oronasal breathing	Uptake efficiencies of URT, LRT, and total RT measured by sampling inspired and expired air from catheter inserted through nose to posterior oropharynx. Uptake efficiencies computed from peak plateau concentrations on inspiration and expiration. Inspiratory URT uptake efficiencies averaged 40%; inspiratory plus expiratory LRT uptake efficiencies averaged 92%. Small but significant decreases in URT and LRT uptake efficiencies with increasing f. No effect of concentration on uptake. Uptake efficiency of mouth relatively greater than nose by about 10%.	Gerrity et al. (1988)
In vivo	Human	Total RT, URT, LRT	V_T = 1,239-1,650 mL f = 25-35 bpm $\dot{V}_E$ = 41 L/min	Twenty healthy male subjects exposed to 0.4 ppm O_3 while exercising at $\dot{V}_E$ of 41 L/min for 1 h. Uptake efficiencies of URT, LRT, and total RT measured at beginning and at end of exposure by method of Gerrity et al. (1988). Subjects mouth-breathed only. Uptake efficiencies computed as mass fractions. URT inspiratory efficiency was 40% and did not change during exposure. LRT efficiency dropped from 68% to 62% during exposure. LRT decrease correlated with drop in V_T. Cumulative dose of O_3 to LRT was predictive of V_T drop.	Gerrity and McDonnell (1989); Gerrity et al. (1989); Gerrity et al. (1994)
In vitro	Pig Sheep	Trachea	$\dot{V}$ = 50-200 mL/s	Unidirectional O_3 uptake efficiencies of trachea decreased with increasing flow from 0.5 to 0.15 for the sheep and 0.12 for the pig. Mass transfer coefficients generally were independent of flow.	Ben-Jebria et al. (1991)

Table 8-1 (cont'd). Experimental Studies on Ozone Dosimetry[a]

Type of Study	Species (Strain)	Uptake	Breathing Patterns	Results	Reference
In vitro	N/A	N/A	N/A	Ozonolysis studies on various unsaturated fatty acids, rat erythrocyte ghost membranes and rat BAL. Dominant processes are the production of aldehydes and peroxides due to reactions between O_3 and olefins.	Pryor et al. (1991)
In vivo	Human	20-200 mL depth into total RT	V_T = 500 mL $\dot{V}_I$ = 250 mL/s	Uptake efficiencies by measuring recovery of O_3 boluses delivered at 20 mL increments into lung to depth of 200 mL. At deepest depth, only 6% of O_3 could be recovered. Ozone uptake by conducting airways larger than predicted by Miller et al. (1985).	Hu et al. (1992b)
In vivo	Human	20-200 mL depth into total RT	V_T = 500 mL $\dot{V}_I$ = 150, 250, 500, 750, 1,000 mL/s	Same technique as Hu et al. (1992b), but investigating flow effects. Increasing flow caused marked shift of delivered O_3 toward the periphery of the conducting airways (i.e., the greater the inspiratory flow, the greater the amount of O_3 delivered to the lung periphery), where it is available for absorption. Mass transfer coefficients in upper airways independent of flow, but in conducting airways they increase proportional to flow. Lung liquid lining mass transfer coefficient computed to be 1.4 cm/s in the URT, falling to 0.17 cm/s in the respiratory airways. Reaction rate constant between O_3 and the lung liquid lining was computed as 7.3×10^6/s in the URT, falling to 8.2×10^5/s in the distal conducting airways.	Hu et al. (1994)
In vivo	Human	20-200 mL depth into total RT	V_T = 500 mL $\dot{V}_I$ = 250 mL/s	Comparison of O_3 bolus uptake between oral and nasal routes. Nose was found to be 30% more efficient at removing O_3 from the air stream than the mouth.	Kabel et al. (1994)
In vitro	Rat (S-D)	Lung	V = 2.71 mL f = 50-103 bpm FRC = 4 and 8 mL	Perfused and nonperfused rat lungs ventilated with 1 ppm O_3. Uptake efficiency of lungs dropped from 95% at 50 bpm to about 50% at 103 bpm. No change in uptake efficiency when lungs inflated from FRC = 4 to 8 mL.	Postlethwait et al. (1994)
In vivo	Human	Total RT, URT, trachea, mainstem bronchi	V_T = 810 mL f = 12 bpm $\dot{V}_I$ = 320 mL/s	O_3 uptake efficiencies in conducting airway structures determined by sampling air from anatomical sites ranging from the vocal cords to bronchus intermedius in 10 subjects undergoing transnasal bronchoscopy while being exposed to 0.4 ppm O_3. Total RT uptake efficiency was 91%. Uptake efficiencies of mouth-vocal cords: 17.6%; vocal cords-upper trachea: 12.8%; upper trachea-main bifurcation carina: 11.5%; main bifurcation carina-bronchus intermedius: 0%.	Gerrity et al. (1995)
In vivo	Human	Total RT	V = 593-642 mL f = 16 bpm $\dot{V}_E$ = 9.2-9.8 L/min Nasal and oral breathing	Total RT uptake efficiency measured using the same technique (Wiester et al., 1987; Wiester et al., 1988) applied to rats. During nasal breathing, total RT uptake efficiency was 73%. During oral breathing, total RT uptake efficiency was 76% and significantly higher than with nasal breathing.	Wiester et al. (1996)

[a]See Appendix A for abbreviations and acronyms.

O_3 uptake was determined by mass balance. The uptake was the difference in mass in the upstream air and in the air downstream of the rat. Total RT O_3 uptake efficiency of approximately 40% was measured and was independent of O_3 concentration. During data acquisition, the animals had an average tidal volume (V_T) of about 2.8 mL, an average breathing frequency (f) of about 150 breaths per minute (bpm), and an average minute ventilation ($\dot{V}_E$) of about 400 mL/min. This study was followed by another (Wiester et al., 1988) in which total RT uptake was measured in three strains of rats and in the guinea pig. Specifically, Fischer 344 (F344), S-D, and Long-Evans rats and Hartley guinea pigs were exposed for 1 h to 0.3 ppm O_3; F344 rats also received a 0.6-ppm exposure. All rats in the Wiester et al. (1988) study had f's between 112 and 132 bpm, V_Ts between 2.4 and 2.8 mL, and $\dot{V}_E$s between 299 and 364 mL/min. The guinea pigs had a V_T of 2.4 mL (not different from rats), an f of 77 bpm, and a $\dot{V}_E$ of 188 mL/min. Uptake was measured as in the previous experiment (Wiester et al., 1987). Total RT uptake of O_3 was species-independent and averaged 47%; this was higher than in the previous study because a different calculation method for fractional uptake was used. As in the first experiment, exposure concentration did not affect uptake. Wiester et al. (1987) corrected all flows used in uptake calculations for body temperature and relative humidity. In their later work, however, they found that this correction was not warranted, resulting in a slightly higher computed O_3 uptake efficiency (Wiester et al., 1988).

These data are in disagreement with the model predictions of Overton et al. (1987), who made predictions of O_3 uptake in two different rat anatomical lung models (Kliment, 1973; Yeh et al., 1979). Simulations were conducted in both anatomical models, varying f and V_T at fixed $\dot{V}_E$; functional residual capacity (FRC) also was varied as a fraction of total lung capacity (TLC). The Kliment (1973) anatomical lung model gave consistently high predictions for uptake when compared with actual data. The predictions using the Yeh et al. (1979) model came closer. Total RT uptakes (not including the head) for the Yeh et al. (1979) model were predicted to range between 46 and 60% at $f = 154$ bpm and $V_T = 1.25$ mL and between 70 and 80% for $f = 81$ bpm and $V_T = 2.4$ mL. However, when the fact that these predictions do not include the head of the animal is considered, it is evident that the model predictions overestimate the total RT uptake in rats. The question is whether the measurements are accurate or whether there is a problem with the model formulation. The data of Postlethwait et al. (1994), presented below, suggested that the data of Wiester et al. (1987, 1988) may be reasonable. The Postlethwait et al. (1994) data in the excised rat lung suggested a clear inverse dependence of lung uptake on f. At a V_T of 2.5 mL, the O_3 uptake efficiency of the excised lung fell from nearly unity at $f = 50$ bpm to almost 50% at $f = 100$ bpm. For extrapolation purposes, a key question here is what should be considered the normal resting f of a rat. Although Wiester et al. (1987, 1988) allowed their rats to acclimate to the plethysmograph by monitoring f and began uptake measurements only after f had plateaued at a minimum, it is still uncertain whether f's of 120 to 150 bpm are reasonable. In a summary of studies of the pulmonary function of rats in response to O_3, Tepper et al. (1993) found typical f's of 100 bpm. Although the models appear to overestimate the O_3 uptake efficiency of the rat RT, the discrepancy is not large, and the near agreement indicates that the O_3 dosimetry models have predictive capability.

In addition to data on the total RT O_3 uptake efficiency, in vivo data on regional O_3 dosimetry in animal models have begun to emerge. Hatch and Aissa (1987) and Aissa and Hatch (1988) first described a method to measure O_3 uptake in animals by exposing them nose-

only, while in a plethysmograph, to O_3 enriched with ^{18}O, a stable isotope of oxygen. After exposure, bronchoalveolar lavage (BAL) fluid and respiratory tract tissue were assayed for excess ^{18}O using isotope ratio mass spectrometry. One problem with this technique is that all of the absorbed ^{18}O cannot be accounted for, thus possibly leading to an underestimation of dose. The technique used by Hatch and Aissa (1987) involves the detection of excess ^{18}O in $^{18}O_3$ reaction products after tissue pyrolysis. Thus, $^{18}O_3$ that is degraded to $H_2{}^{18}O$ or $^{18}O_2$ is lost and cannot be detected in dry tissue. Eight male F344 rats (four previously exposed chronically to O_3 for 1 year to an urban pattern generally consisting of a 0.06-ppm baseline with 1-h daily spikes rising to 0.25 ppm) were exposed for 2 h to 1 ppm of ^{18}O-enriched O_3 (Hatch et al., 1989). During exposure, the rats breathed at 150 bpm and had a V_T of 2.05 mL and a $\dot{V}_E$ of 290 mL/min. After exposure, the lung, trachea, and head were analyzed separately for ^{18}O. Overall, the animals took up 54.3% of inspired O_3. Although this value of O_3 uptake efficiency is higher than that found by Wiester et al. (1987, 1988), considering the fact that the coefficient of variation for O_3 uptake efficiency measurements is around 20% in all studies, the result of Hatch and Aissa (1987) is consistent with the data of Wiester et al. (1987, 1988). Of the O_3 taken up by the animals, 49.6% was taken up by the head, 6.7% by the larynx/trachea, and 43.6% by the lungs. By assuming equal uptake efficiencies by compartments on inspiration and expiration, inspiratory uptake of O_3 by these regions was computed. It was determined that the rat nasopharynx (NP) had an inspiratory efficiency of 17.4%, and that the larynx/trachea removed 2.7% of the remaining O_3.

This technique recently has been extended to humans. Hatch et al. (1994) showed that when human subjects were exposed to 0.4 ppm $^{18}O_3$ while exercising intermittently at $\dot{V}_E$ = 60 L/min for 2 h, the amount of recovered ^{18}O in lavagable cells indicated that the human cells incorporated 4 to 5 times the O_3 dose (i.e., concentration of $^{18}O_3$) that was incorported by the BAL cells from rats exposed to 0.4 ppm O_3 for 2 h at rest. Consequently, to compare absorbed $^{18}O_3$ doses between rats and humans using BAL requires the assumption that the amount of lavagable cell membrane available to react with $^{18}O_3$ is comparable between the two species. The difference between rats and humans could be accounted for by the fact that the humans were exercising, whereas the rats were not. However, as was noted above, not all absorbed $^{18}O_3$ can be accounted for.

8.2.3.3 In Vitro Ozone Dosimetry Studies

The use of whole, intact animals to study O_3 uptake is needed to ascertain the actual amounts of O_3 absorbed. However, it is also important to understand some of the more fundamental processes governing O_3 uptake, such as the biochemistry of O_3/liquid and O_3/tissue interactions to determine chemical reaction rates essential to O_3 dosimetry models. Furthermore, the use of intact animals does not allow more precise determinations of the role of physiological parameters on O_3 uptake. For this reason, there have been some limited attempts at utilizing animal tissue explants and whole lungs to study O_3 uptake.

Ben-Jebria et al. (1991) studied O_3 uptake by the trachea of sheep and pigs to investigate mass transfer coefficients. Ozone boluses of 1 ppm were passed through excised tracheae. Tracheae were obtained from a slaughter house 0.5 to 2 h after slaughter, and, although they were kept coated with physiologic saline, they were not maintained at body temperature, possibly resulting in underestimation of in vivo uptake. The lengths and diameters of the pig tracheae were not too different from human tracheal dimensions. The flow dependence of mass uptake and the mass transfer coefficient (K) were determined for

flows between 50 and 200 mL/s. Uptake efficiencies in the pig decreased with increasing flow from about 0.5 to 0.12 and in the sheep decreased from about 0.5 to 0.15. Mass transfer coefficients generally were independent of flow (K = 0.5 cm/s in pigs and 0.35 cm/s in sheep), indicating the lack of dependence of uptake on gas-phase diffusion processes. This contrasts with the conclusion of Aharonson et al. (1974) for the NP of dogs where the investigators observed that the slight inverse dependence of uptake on flow observed by Yokoyama and Frank (1972) leads to the conclusion that the mass transfer coefficient for the NP of the dog should increase with flow, suggesting a role of the boundary layer in limiting diffusion of O_3 to the wall of the NP. The different geometries of a trachea and an NP may account for the differing observations.

A significant feature of the Ben-Jebria et al. (1991) study was the use of a rapidly responding O_3 analyzer. In order to conduct their O_3 uptake studies, Ben-Jebria and Ultman (1989) and Ben-Jebria et al. (1990) developed a rapidly responding O_3 analyzer. The analyzer relies on the reaction of O_3 with alkenes such as ethylene, propylene, cyclohexane, etc. Ten alkenes were tested. Ninety percent step-response times of 130 to 540 ms were achieved with varying degrees of linear response with O_3 concentration. The authors concluded that the best alkene was 2-methyl-2-butene, with optimum 10 to 90% responses of 110 ms and minimum detectable limits of O_3 of 18 ppb. Interference with CO_2, however, was found, requiring measurements of CO_2 to correct the analyzer response.

Postlethwait et al. (1994) used an isolated rat lung preparation to investigate the effects of vascular perfusion, inspired dose, temperature, and distal lung surface area on O_3 absorption by the LRT. Vascular perfusion had little or no effect on uptake efficiency of O_3. When the lung was exposed to 1 ppm O_3 and ventilated with a V_T of 2.5 mL, a FRC of 4 mL, and an f of 50 bpm, uptake efficiency was 95%. As f increased with fixed V_T, uptake efficiency began to drop, reaching nearly 50% at an f of 100 bpm. When the lung temperature was dropped from 37 to 25 °C, uptake efficiency dropped from 95 to 85% at 50 bpm. This drop was exploited to investigate other factors (such as flow, volume, and lung surface area) governing uptake because it moved respiratory tract uptake further below 100%. The observation of a dependence of uptake on temperature indicates that uptake efficiency is chemical-reaction dependent, thus possibly coupling uptake to reaction product formation. Another interesting result from this study was the lack of dependence of uptake on FRC. When FRC was doubled from 4 to 8 mL at 25 °C, fractional O_3 uptake was unchanged. This latter result suggests that O_3 uptake is virtually complete by the time O_3 reaches the alveolar spaces of the lung. Otherwise it would have been expected that the uptake efficiency would have risen with increased FRC.

To further investigate the reactions of O_3 with the lung, Pryor et al. (1991) performed ozonolysis studies of various unsaturated fatty acids (UFAs), rat erythrocyte ghost membranes, and rat BAL. These studies demonstrated significant production of hydrogen peroxide and aldehydes and that production of hydrogen peroxide was due primarily to reactions between O_3 and olefins. The authors concluded that the reaction of O_3 with UFAs in the lung fluid lining and cell membranes produce hydrogen peroxide and aldehydes that may be important mediators in the toxicity of O_3. The quantitative results of these studies led Pryor (1992) to hypothesize about the degree to which O_3 reacts with the liquid lining of the lung and with lung tissue. A simple model calculation was performed using the Einstein-Smoluchowski equation to estimate the half-life of O_3 in bilayers and cell membranes. Pryor (1992) concluded that a substantial fraction of O_3 reacts in the bilayer, and that only in regions of the

lung where the lung lining fluid layer is less than 0.1 μm thick will O_3 penetrate to tissue, and only then will O_3 react in cell membranes before penetrating further. The overall conclusion is that the toxic effects of O_3 may be mediated not just by O_3 directly but by reactive intermediates such as aldehydes and hydrogen peroxide. This raises the question as to the relevant dose of O_3: is it the total dose, the dose to the liquid lining, the tissue dose, or the dose of reactive intermediates delivered to tissue?

8.2.3.4 Human Ozone Dosimetry Studies

Significant progress has been made in the area of human O_3 dosimetry since the previous criteria document (U.S. Environmental Protection Agency, 1986). Studies have been conducted defining total and regional respiratory tract uptake, the dependence of uptake on physiological parameters, and the role of uptake in modulating response.

Gerrity et al. (1988) reported on measurements of O_3 uptake by the extrathoracic airways (airways proximal to the posterior oropharynx) and intrathoracic airways (airways distal to the posterior oropharynx) in 18 healthy, young male volunteers. Ozone uptake was measured by placing a small polyethylene catheter through the nose and positioning the distal tip in the posterior oropharynx. Breath-by-breath samples of O_3 were collected, and the peak plateau concentrations were compared with chamber concentrations. The effects on uptake of O_3 concentration (0.1, 0.2, 0.4 ppm), f (12 and 24 bpm at fixed V_T), and mode of breathing (oral, nasal, and oronasal) were tested. The O_3 analyzer had a moderately rapid response with a 90% response time of 700 ms. Inspiratory V_T ranged between 754 and 848 mL; mean inspiratory flow at 12 bpm was 350 mL/s; at 24 bpm it was 634 mL/s. The authors measured extrathoracic uptake efficiency of O_3 on inspiration at approximately 40% and intrathoracic uptake efficiency (inspiration plus expiration) at approximately 92%. They essentially found no effect of O_3 concentration on uptake (intrathoracic uptake was significantly higher at 0.4 ppm, but the difference was very small). They did find that both intrathoracic and extrathoracic uptake decreased with increasing f (at fixed V_T), falling by about 7% for extrathoracic uptake and by about 3% for intrathoracic uptake when f increased from 12 to 24 bpm. The finite response time of the analyzer may have affected the results at the 24 bpm f by overestimating extrathoracic uptake and underestimating intrathoracic uptake. However, because uptake was defined relative to plateaus of concentration, the response time of the analyzer was adequate to reach a plateau in the 1.2-s inspiratory time at 24 bpm. It is important to note here that, when utilizing the data from this study to compare with other studies and models, the uptake efficiencies measured are comparable to steady-state unidirectional measurements of uptake. Another feature is that Gerrity et al. (1988) consistently measured a small, non-zero plateau of O_3 on expiration. This plateau is not consistent with the suggestion from models of O_3 uptake and the data of Postlethwait et al. (1994) (nor with the later work of Gerrity et al. [1995] that is presented below) that no O_3 should be washed out from lung volumes beyond the conducting airways. This observation of Gerrity et al. (1988) may have been an artifact of the manner in which O_3 was measured by sampling from the posterior oropharynx. There may have been entrainment of O_3 in the pharyngeal airspaces that was washed out after expiration of dead-space air. Regardless, the concentration of O_3 exhaled from the alveolar phase of washout was very low.

One of the most startling results from the work of Gerrity et al. (1988) was the finding that there was only a small, but statistically significant, difference between uptake by the nose and by the mouth. The mouth had approximately 10% greater uptake efficiency than

the nose. The combined oronasal passage had an uptake efficiency greater than the nose by another 8%. This suggests that persons who breathe nasally are at no less risk than persons who breathe oronasally. Adams et al. (1989) investigated this possibility by comparing functional responses in subjects acutely exposed to O_3 while breathing either orally or oronasally. Healthy subjects were exposed on five separate days to 0.4 ppm O_3. In the first four exposures, subjects were exposed by face mask (with or without nose clip) for 30 min at an exercise level of 75 L/min or for 75 min at exercise level of 30 L/min. The fifth exposure was for 30 min at 75 L/min, with exposure through a mouthpiece. There were no differences in pulmonary function response (forced expiratory volume in 1 s [FEV_1], forced vital capacity [FVC], or forced expiratory flow) with face-mask exposure among all experimental groups (i.e., no nose clip, $\dot{V}_E$, or time effect). Pulmonary function response was, however, greater with a mouthpiece. Adams et al. (1989) speculated that the greater response with the mouthpiece was due to O_3 scrubbing by the face mask or by facial hair. It also may have been due to different oral configurations imposed by a mouthpiece. Hynes et al. (1988) also investigated whether functional responses were affected by the mode of breathing. Healthy subjects were exposed to 0.4 ppm O_3 for 30 min in an exposure chamber. On two different occasions, each subject breathed either through the nose or the mouth exclusively. There was no difference in pulmonary function response between these two routes of exposure. Taken together, the studies of Adams et al. (1989) and Hynes et al. (1988) are consistent with the observations of Gerrity et al. (1988) on the equal efficiency of all routes of breathing for extrathoracic O_3 scrubbing.

This study was followed by another study (Gerrity and McDonnell, 1989; Gerrity et al., 1989, 1994) in which the relationship between O_3 uptake and functional response was investigated. Healthy subjects were exposed to 0.4 ppm O_3 for 1 h while exercising continuously at 40 L/min. Ozone uptake was measured at the beginning and at the end of exposure while the subjects were still exercising, using the technique of Gerrity et al. (1988). In contrast to the work of Gerrity et al. (1988), uptake was computed in this study by integrating concentration times flow instead of using peak plateau measurements. Also, in this work, the 90% response time of the analyzer was 1.2 s (compared with 0.7 s in the previous work). The authors found that about 40% of the inspired O_3 was taken up by the URT (i.e., the same as the extrathoracic airways described in Gerrity et al., 1988) during inspiration, and that this did not change during exposure. Total RT uptake efficiency was approximately 80%, and it did not change during exposure. However, LRT (i.e., the intrathoracic airways described in Gerrity et al., 1988) uptake efficiency fell during exposure from 68 to 62% and was correlated with the O_3-induced fall in V_T (V_T fell from 1,650 to 1,239 mL; f increased from 25.2 to 34.8 bpm; inspiratory flow fell from 1,506 to 1,357 mL/s; $\dot{V}_E$ increased slightly from 40.8 to 40.9 L/min), suggesting that the V_T reduction may have a protective effect on dose delivered to the periphery of the lung. It is not likely that the finite analyzer response time affected uptake measurements. Evidence of this is the lack of dependence of URT uptake on changes in V_T or f. The low values for uptake in the LRT may have been due to an artifact from the relatively slow response time of the analyzer, which was approximately equal to inspiratory and expiratory times. As a check on their results, the authors compared their data with the previous work of Gerrity et al. (1988) by computing uptake by the original technique using peak plateau concentrations. When that was done, the URT uptake efficiencies were 17 and 22% at the beginning and end of exercise, respectively, and the LRT efficiencies were 96 and 92% at the beginning and end of exercise, respectively. The URT change computed this

way was not significant, but the LRT efficiency drop was. When viewed at in this manner, the data from this experiment are consistent with those from the previous experiment.

Because there is a need to compare human O_3 uptake data with rat O_3 uptake data, it is essential that there be confidence in the reliability of the different approaches. To help establish the comparability of techniques, Wiester et al. (1996) measured total RT uptake in humans using a similar, although obviously scaled-up, system to that used for rats (Wiester et al., 1987, 1988). Healthy subjects breathed 0.3 ppm O_3 while seated in an exposure chamber; their faces were placed in a sealed face mask. The face mask was attached to a large tube through which chamber air was circulated with a pump at a rate of 40 L/min. Upstream and downstream O_3 concentrations were measured continuously, as was ventilation with an induction plethysmograph. Subjects breathed at rest, either through nose or mouth (average f = 16 bpm, V_T = 598 to 642 mL, $\dot{V}_E$ = 9.2 to 9.8 L/min). While nose breathing, 73% of inspired O_3 was taken up by the total RT, and, while mouth breathing, 76% of inspired O_3 was taken up, which was significantly higher than that found with nose breathing. This difference is probably not, however, biologically significant. Significant negative correlations between f and uptake in both mouth- and nose-breathers were found, similar correlations were found with $\dot{V}_E$, but no correlations were found between uptake and V_T or any other measure of breathing pattern or pulmonary function.

The observations in Wiester et al. (1996) of a slight increase of total RT uptake efficiency with oral breathing and the inverse correlation of total RT uptake efficiency with f are consistent with those of Gerrity et al. (1988). Furthermore, the data on total RT uptake are consistent overall with that of Gerrity et al. (1988, 1994). The data from Gerrity et al. (1988) reporting total RT uptake efficiencies of about 95% were based on minimum plateau measurements, thus reflecting uptake during steady-state flow conditions, as opposed to the cyclical conditions of actual breathing. The data of Gerrity et al. (1994), on the other hand (in which total RT efficiencies of 80% were reported), were obtained by integrating the product of concentration and flow, thus more accurately reflecting the actual mass uptake of O_3 during cyclical breathing when Gerrity et al. (1994) computed uptake using the methodology from Gerrity et al. (1988), they found that the total RT uptake measurements were comparable. Thus total RT mass uptake efficiencies at rest of 80% are not unreasonable.

Hu (1991), Hu et al. (1992b, 1994), and Ultman et al. (1993) took a different approach to measuring respiratory tract uptake of O_3. They exploited the development of a rapid responding O_3 analyzer (Ben-Jebria and Ultman, 1989; Ben-Jebria et al., 1990) to measure the recovery of small boluses of O_3 delivered to different volumetric depths of the RT. Ozone uptake was measured in a set of four experiments. In the baseline experiments, absorption of O_3 boluses was measured in healthy male subjects at rest. The O_3 boluses were 10 mL in size, with a peak concentration of 3 ppm. The O_3 analyzer characteristics were sample flow of 400 mL/min, 2-methyl-2-butene as reactive alkene, 10 to 90% step-response time of 110 ms, and lower detection limit (18 ppb). In the baseline experiments, the V_T was 500 mL, and the inspiratory and expiratory flow rates were 250 mL/s. In a complete set of measurements, bolus recovery was examined for penetrations of 10 to 200 mL, in 10 mL increments. In the second set of experiments, the effects of flow were measured by measuring bolus recovery as a function of penetration depth for flows of 150, 250, 500, 750, and 1,000 mL/s at a fixed V_T of 500 mL. In a third set of experiments, bolus recovery was measured as a function of penetration depth at a flow of 250 mL/s and a V_T of 500 mL; the bolus delivered to a rubber mouthpiece or to a nasal cannula was compared, thereby examining

potential uptake differences between the two pathways. In the fourth set of experiments, the effects of O_3 concentration on uptake were determined by delivering boluses with peak concentrations of 0.5, 1.0, 2.0 and 4.0 ppm. The latter experiments were conducted because acute studies in isolated dog airways showed that absorption efficiency was inversely related to inhaled concentration between 0.1 and 20 ppm (Vaughan et al., 1969; Yokoyama and Frank, 1972). However, later experiments in guinea pigs, rabbits (Miller et al., 1979), and humans (Gerrity et al., 1988) showed a lack of concentration dependence, implying a linear relationship between concentration and dose. The dependence or lack of dependence of uptake efficiency on O_3 concentration provides information on the order of reactions of O_3 with lung fluid lining and tissue. Under steady-state conditions, concentration independence of uptake efficiency suggests that first-order processes play a role. However, because O_3 absorption is coupled to both interfacial transfer (gas-phase to solute O_3) and subsequent reaction, at face value, the conclusion cannot be reached that saturated absorption rates are solely due to saturation of the reaction components.

In all four experiments, Hu and colleagues computed the first three moments of the inspired and expired bolus distributions with respect to volume. The zeroth moment of a bolus is the O_3 mass contained in the bolus. Thus the zeroth moments of the inspired and expired boluses were used to compute O_3 uptake efficiency (η; or absorbed fraction), breakthrough volume (the mean volume of the exhaled bolus), and bolus dispersion. The first moment of a bolus is its mean volumetric position. The first moment on inspiration gives the penetration volume (V_p), and the first moment on expiration gives the breakthrough volume (V_B). In the absence of any O_3 uptake, a longitudinally mixed bolus should have $V_B = V_p$. The second moment of a bolus is its variance. The difference in variance between the expired and inspired bolus (σ^2) is a measure of gas mixing, or dispersion, in the lung.

In the baseline experiments, the breathing pattern was a resting pattern with a V_T of 500 mL and an average inspiratory flow of 250 mL/s. These experiments were performed on nine male subjects and showed that almost all O_3 was absorbed beyond a penetration depth of 180 mL. Only about 6% of inhaled O_3 was recovered at the 180 mL penetration depth, and, beyond that depth, it was very difficult to obtain an accurate measurement of recovery. The investigators also found that V_B was greater than V_p at penetration depths less than 100 mL, after which V_B leveled out at a constant value. Dispersion was insensitive to penetration depth. An important finding of the baseline experiment was that at quiet resting ventilation, about 50% of the O_3 mass in a bolus inhaled through the upper airways is taken up by the upper airways. To compare these data with results of Gerrity et al. (1988), it is necessary to assume that inspiratory and expiratory uptake efficiencies are equal. Then the unidirectional uptake efficiency of the upper airways to a depth comparable to that at which Gerrity et al. (1988) positioned their sampling catheter is about 30%, which is approximately 25% less than the 40% results of Gerrity et al. (1988). This difference might be due to the presence of a mouthpiece in the experiments of Hu and colleagues, which could reduce the uptake efficiency of the oral pathway. The functional response data of Adams et al. (1989) suggest that this might be the case.

The flow experiments showed that there was a general right shifting of the η - V_p curves with increasing flow (i.e., increasing flow causes a deeper penetration of O_3 into the lung with lower fractional uptake by the conducting airways). Eventually, all of the O_3 is still absorbed. Breakthrough volume showed a similar pattern at all flows (i.e., greater than penetration volume at small V_p but flattening out at larger V_p). As flow increased, the level of

the plateau increased. Dispersion, although constant as a function of V_p at all flows, increased linearly with increasing flow.

The studies of Hu and colleagues investigating the role of exposure route in modulating O_3 uptake efficiencies reported that the nose absorbed approximately 30% more than the mouth. This result is at variance with the findings of Gerrity et al. (1988), which indicate that there was only a slightly higher uptake by the oral pathway when compared with the nasal pathway. Gerrity et al. (1988) studied uptake by the two pathways without the use of a mouthpiece or any other delivery system. Subjects were free to breathe naturally. It is possible that the use of mouthpieces and nasal canulas in the studies of Hu and colleagues caused artifacts, resulting in their findings for nasal and oral uptake efficiencies. The study of Adams et al. (1989) supports this, showing enhanced pulmonary function response to O_3 during a mouthpiece exposure compared to face-mask/oral exposure. Finally, the concentration-dependence studies showed that uptake efficiency was not affected by the concentration of inspired O_3 between 0.3 to 4 ppm, implying that O_3 uptake is governed by linear processes.

One of the very unique features of the approach to measuring uptake efficiency taken by Hu and colleagues is that the O_3 bolus recovery data can be used to derive local mass transfer coefficients for the conducting airways. Regional mass transfer coefficients derived experimentally in this way can then be used as input into mathematical model simulations, thereby potentially leading to more accurate models of O_3 dose to the RT.

Hu and colleagues define the parameter Ka (per second) as one that is suitable to characterize local O_3 absorption. It is the product of the overall K (centimeters per second), which reflects the combined contribution of diffusion and chemical reaction to uptake, and the local surface/volume ratio (a; per centimeter). From the □ □ V_p curves, these investigators derived values for Ka. Thus, the experimentally derived mass transfer coefficients depended on assumptions about airway anatomy and morphology. As a result of the various experiments, these investigators found a number of important properties of Ka:

- The proximal subcompartment of the nose has a Ka that is 70% larger than the Ka for the proximal mouth compartment (see, however, the comment made above regarding the nose/mouth differences).
- Ka's in the upper airway compartment were between 1.20 and 2.24/s and relatively insensitive to flow, indicating that diffusion resistance of O_3 through the gas boundary layer is much less than through the mucus film. These data also are consistent with the pig and sheep tracheae experiments of Ben-Jebria et al. (1991).
- In the proximal and distal conducting airway subcompartments, $(Ka)^{-1}$ was linearly related to $(flow)^{-1}$, suggesting that the gas-phase absorption rate constant is directly proportional to flow. In lower airways, therefore, diffusion resistance of the gas boundary layer is important. Hu and colleagues concluded that the gas boundary layer contributes 80 to 90% of the overall diffusion resistance in the central airway compartment.
- The mass transfer coefficient in the lung liquid lining is estimated to fall from 1.4 cm/s in the URT to 0.17 cm/s in the respiratory airways.
- The chemical reaction rate between O_3 and the lung liquid lining was estimated to be 7.3×10^6/s, 2.3×10^6/s, and 8.2×10^5/s in the upper airways, proximal conducting airways, and distal conducting airways, respectively.

- The overall mass uptake coefficients determined in the work of Hu (1991) are significantly higher than those used in the model of Miller et al. (1985). If the mass transfer coefficients in the model are adjusted upward, the total uptake efficiency would be higher than measurements have shown, requiring a downward adjustment of pulmonary region mass transfer coefficients.

Gerrity et al. (1995) took a somewhat more conventional approach in an attempt to acquire regional information on O_3 absorption in the human RT. Healthy subjects underwent transnasal bronchoscopy while in an exposure chamber in which 0.4 ppm O_3 was present. Subjects were asked to breathe at 12 bpm. Inspired and expired air was sampled through a Teflon catheter that had been passed through the biopsy channel of the bronchoscope and positioned approximately in the center of the airway lumen. The air was drawn into a rapid response O_3 analyzer (Ben-Jebria and Ultman, 1989; Ben-Jebria et al., 1990) with a 90% response time of 250 mL/s while using ethylene as the reactive alkene. Average V_T was 810 mL, and average inspiratory flow was 320 mL/s. Air was sampled for five breaths from above the vocal cords, at the entrance to the trachea, above the main bifurcation carina, and midway through the bronchus intermedius. Flow was measured simultaneously by a pneumotach attached to a simple cylindrical mouthpiece. Before and after each measurement, a set of samples from the mouth was collected for reference. Uptake was defined as the fraction of O_3 mass lost across any anatomical segment; mass was determined by integrating the product of O_3 concentration and flow. By way of comparison with the other human O_3 uptake studies, Gerrity et al. (1994) found that total RT uptake of O_3 measured in this fashion was 91%. This is higher than the resting data of Wiester et al. (1996); however, the average V_T in this study of 810 mL, compared with the 600 mL VT reported by Wiester et al. (1996) may account for this difference. When Gerrity et al. (1995) computed the unidirectional uptake efficiencies between the mouth and the various sampling sites, they found that 17.6, 27.0, 35.5 and 32.5% of the O_3 passing into the mouth is taken up by structures up to the vocal cords, the upper trachea, the main bifurcation carina, and the bronchus intermedius, respectively. They also computed the unidirectional uptake efficiencies across individual airway segments: 17.6% between the mouth and just above the vocal cords, 12.8% from above the vocal cords to the upper trachea, 11.5% from the upper trachea to the main bifurcation carina, and essentially zero between the carina and bronchus intermedius. The uptake between the mouth and just above the vocal cords is considerably lower than that measured earlier by Gerrity et al. (1988), even considering the fact that, in the earlier study, peak plateaus were used. As has been noted earlier, it is possible that the mouthpiece played a role in reducing the uptake efficiency of the mouth. The uptake efficiency of O_3 across the trachea is in line with the data from sheep and porcine tracheae at the higher flow rates (Ben-Jebria et al., 1991). The present data are also consistent with the bolus uptake data of Hu (1991) and colleagues, which also were acquired with a mouthpiece. When the O_3 bolus data are used to compute unidirectional uptake efficiencies (assuming that the segmental efficiencies are the same on inspiration and expiration), the Hu et al. (1992b) data yield uptake efficiencies of 21, 36, 44, and 46% between the mouth and the vocal cords, the upper trachea, the main bifurcation carina, and the bronchus intermedius, respectively. The O_3 bolus data are, therefore, in good accord with the data of Gerrity et al. (1995). The measured uptake efficiencies across airway segments clearly appear to be higher than those predicted by the model of Miller et al. (1985). If higher uptake coefficients in the conducting airways were used in the model of Miller et al. (1985), the model would overestimate total RT O_3 uptake.

To adjust for this, pulmonary uptake coefficients would have to be reduced. Unfortunately, the data of Hu and associates cannot provide information beyond the conducting airways.

Gerrity et al. (1995) also measured O_3 washout volumes (i.e., the expired volumes required to cause a specified drop in O_3 concentration). This type of data provides insight into the location of major sites of O_3 uptake. At the mouth, the 90% washout volume was 142 mL, and, at the upper trachea, the 90% washout volume was 62 mL. By the time the entire anatomical dead space of the lungs was washed out, the O_3 concentration had fallen to zero (Gerrity et al., 1995). It is unclear whether the absence of recovered O_3 after washout of the conducting airways was due to O_3 not penetrating beyond the conducting airways or to all of the O_3 that penetrated beyond the conducting airways being absorbed. The latter possibility is more likely based on the observations of Hu (1991).

In assessing the work of Gerrity et al. (1995), it is significant to note that these investigators measured expired plateaus of O_3 concentration that were zero. This contrasts with the earlier work of Gerrity et al. (1988, 1994) in which a non-zero expiratory plateau was observed. The non-zero expiratory plateau may have been due to a number of factors that are unclear. Because ethylene was the reacting alkene in all cases, it is unlikely that interference with other gases such as carbon dioxide (CO_2) was responsible. Another possibility is that O_3 in the early expiratory phase became entrained in the posterior oropharynx and persisted for the duration of expiration.

8.2.3.5 Intercomparison of Ozone Dosimetry Studies

The previous sections emphasized the methods and results of individual experimental studies on O_3 dosimetry. This section will focus on comparisons of the in vivo studies with each other and will draw on these comparisons to arrive at conclusions regarding the utility of these data for extrapolation purposes. The discussion will be divided into three sections, focusing on total RT uptake efficiency, unidirectional URT uptake efficiency, and LRT uptake efficiency.

There are two categories that distinguish various data sets among each other. The first category is the mouth/nose category listed in the Tables 8-2 to 8-4. Studies indicated by "M" or "N" were performed with unencumbered breathing by either oral (M) or nasal (N) breathing. *Unencumbered* indicates the absence of a mouthpiece or canula. Data listed as "M/N" are pooled from data encompassing oral and nasal breathing. Data shown as "Mouthpiece" or "Nasal canula" are acquired using these devices to deliver the O_3 to the animal or human subject, or to measure flow.

The second category is the method used to compute O_3 uptake efficiency. There are essentially two methods. One method, referred to as the steady-state method, relies on measuring the loss of O_3 from a steady air flow moving across an anatomical structure. An example is the data of Yokoyama and Frank (1972) in which a constant flow of ozonated air through the URT of a dog was maintained by a tracheal canula attached to a pump. Uptake efficiency was measured by changes in equilibrium O_3 concentration. Another example is the study of Gerrity et al. (1988), which used the steady-state method by measuring the peak inspiratory and minimum expiratory O_3 concentrations through a catheter in the posterior pharynx. These measurements were compared to the ambient chamber concentration to obtain uptake efficiencies of the URT and LRT. The second method is referred to as the non-steady state method. This method uses the integration of the product of flow and O_3 concentration to compute O_3 masses that, in turn, are used to compute

Table 8-2. Total Respiratory Tract Uptake Data[a]

Species	Mouth/Nose	Method	V_T(mL)	Inspiratory Flow (mL/s)	f (bpm)[b]	F_t	Reference
Human	M	Steady	832	509	18	0.97	Gerrity et al. (1988)[c]
Human	N	Steady	754	456	18	0.96	Gerrity et al. (1988)
Human	M/N	Steady	832	350	12	0.97	Gerrity et al. (1988)
Human	M/N	Steady	778	634	24	0.96	Gerrity et al. (1988)
Human	M	Non-steady	1,650	1,360	25	0.88	Gerrity et al. (1994)[c]
Human	M	Non-steady	1,239	1,360	35	0.87	Gerrity et al. (1994)
Human	M	Steady	1,650	1,350	25	0.97	Gerrity et al. (1994)
Human	M	Steady	1,239	1,360	35	0.95	Gerrity et al. (1994)
Human	Mouthpiece	Non-steady	825	330	12	0.91	Gerrity et al. (1995)
Human	M	Non-steady	631	539	16	0.76	Wiester et al. (1996)
Human	N	Non-steady	642	514	16	0.73	Wiester et al. (1996)
Human	Mouthpiece	Non-steady	500	250	15	0.86	Hu et al. (1992b)
Human	Mouthpiece	Non-steady	1,000	250	7.5	0.93	Hu et al. (1992b)
Rat (F344)	N	Non-steady	2.8	12.2	118	0.44	Wiester et al. (1988)
Rat (S-D)	N	Non-steady	2.4	9.6	123	0.46	Wiester et al. (1988)
Rat (Long-Evans)	N	Non-steady	2.7	12.3	132	0.48	Wiester et al. (1988)
Rat (F344)	N	Non-steady	2.6	11.3	113	0.54	Hatch et al. (1989)
Guinea pig	N	Non-steady	2.4	7.5	77	0.53	Wiester et al. (1988)

[a]See Appendix A for abbreviations and acronyms. M = mouth exposure by natural breathing; N = nasal exposure by natural breathing; M/N = pooled data from mouth and nasal exposure; Mouthpiece = exposure by mouthpiece; Steady = uptake computed during constant unidirectional flow; Non-steady = uptake computed by integration during cyclic breathing; F_t = total RT uptake.
[b]f is either measured or is computed from reported flows and volumes.
[c]Total RT uptake reported by Gerrity et al. (1988) and Gerrity et al. (1994) did not include the contribution from URT uptake efficiency during expiration. The data include an expiratory URT contribution, assuming it equals inspiratory URT uptake efficiency.

uptake. The studies of Wiester et al. (1988) in rodents, and Wiester et al. (1996) in humans are examples of this technique, as is the study of Gerrity et al. (1994).

Total Respiratory Tract Uptake Efficiency

Table 8-2 provides a summary of in vivo data in all animal species of respiratory tract O_3 uptake efficiency (F_t). The data reported for the studies of Gerrity et al. (1988, 1994) have been adjusted from the published values to account for the fact that the F_t cited in those papers did not include uptake in the URT on expiration. To make the adjustment, the URT uptake efficiency on expiration was assumed to equal the inspiratory uptake efficiency. The F_t data listed for the study of Hu et al. (1992b) were derived from their bolus recovery data by integrating the data over the desired V_T. Because Hu et al. (1992b) could not recover boluses from a depth greater than 220 mL, it was assumed that any bolus delivered

Table 8-3. Unidirectional Upper Respiratory Tract Uptake Efficiency Data[a]

Species	Mouth/Nose	Method	Inspiratory Flow (mL/s)	f (bpm)[b]	F_{urt}	Reference
Human	M	Steady	509	18	0.40	Gerrity et al. (1988)
Human	N	Steady	456	18	0.43	Gerrity et al. (1988)
Human	M/N	Steady	350	12	0.41	Gerrity et al. (1988)
Human	M/N	Steady	634	24	0.38	Gerrity et al. (1988)
Human	M	Non-steady	1,360	25	0.37	Gerrity et al. (1994)
Human	M	Non-steady	1,360	35	0.41	Gerrity et al. (1994)
Human	M	Steady	1,360	25	0.16	Gerrity et al. (1994)
Human	M	Steady	1,360	35	0.22	Gerrity et al. (1994)
Human	Mouthpiece	Non-steady	337	12	0.18	Gerrity et al. (1995)
Human	Mouthpiece	Non-steady	250	15	0.30	Ultman et al. (1993)
Human	Mouthpiece	Non-steady	250	15	0.47	Ultman et al. (1993)
Dog (beagle)	Nasal canula	Steady	83.3	N/A[c]	0.72	Yokoyama and Frank (1972)
Dog (beagle)	Nasal canula	Steady	667	N/A	0.37	Yokoyama and Frank (1972)
Dog (beagle)	Mouthpiece	Steady	83.3	N/A	0.34	Yokoyama and Frank (1972)
Dog (beagle)	Mouthpiece	Steady	667	N/A	0.12	Yokoyama and Frank (1972)
Rat (F344)	N	Non-steady	11.3	113	0.17	Hatch et al. (1989)
Guinea pig	N	Steady	2.7	N/A	0.62	Miller et al. (1979)
Rabbit	N	Steady	16.7	N/A	0.41	Miller et al. (1979)

[a]See Appendix A for abbreviations and acronyms. M = mouth exposure by natural breathing; N = nasal exposure by natural breathing; M/N = pooled data from mouth and nasal exposure; Mouthpiece = exposure by mouthpiece; Nasal canula = exposure by nasal canula; Steady = uptake computed during constant unidirectional flow; Non-steady = uptake computed by integration during cyclic breathing; F_t = total RT uptake.
[b]f is either measured or is computed from flows and V_T.
[c]N/A = not applicable.

to a depth greater than 220 mL was absorbed completely. The derivation of F_t from the bolus data was done for V_Ts of 500 and 1,000 mL.

To assess the consistency of the data, it is useful to examine it as a function of flow. Figure 8-1 shows F_t as a function of inspiratory flow for all human studies. The F_t from the bolus recovery data of Hu et al. (1994) are shown for V_Ts of 500, 1,000, and 1,500 mL. An overview of the data suggests that, with respect to F_t, there is good agreement among the various experimental methods for humans. The data clearly show that F_t decreases with increasing flow and increases with increasing V_T, both of which are qualitatively consistent with model predictions.

One observation is quite prominent: the rat data of Wiester et al. (1988) and Hatch et al. (1989) (not shown in Figure 8-1) are considerably lower than the human data. Even if it is assumed that the rats were breathing up to three times resting ventilation

Table 8-4. Lower Respiratory Tract Uptake Efficiency Data[a]

Species	Mouth/Nose	Method	V_T (mL/s)	Inspiratory Flow (mL/s)	f (bpm)[b]	F_{irt}	Reference
Human	M/N	Steady	832	350	12	0.93	Gerrity et al. (1988)
Human	M/N	Steady	778	634	24	0.89	Gerrity et al. (1988)
Human	M	Non-steady	1,650	1,360	25	0.68	Gerrity et al. (1994)
Human	M	Non-steady	1,239	1,360	35	0.62	Gerrity et al. (1994)
Human	M	Steady	1,650	1,360	25	0.96	Gerrity et al. (1994)
Human	M	Steady	1,239	1,360	35	0.92	Gerrity et al. (1994)
Human	Mouthpiece	Non-steady	844	337	12	0.95	Gerrity et al. (1995)
Human	Mouthpiece	Non-steady	500	250	15	0.78	Hu et al. (1992b)
Human	Mouthpiece	Non-steady	1,000	250	7.5	0.89	Hu et al. (1992b)
Dog (beagle)	N/A[c]	Non-steady	168	112	20	0.87	Yokoyama and Frank (1972)
Dog (beagle)	N/A	Non-steady	168	168	30	0.83	Yokoyama and Frank (1972)
Rat (F344)	N	Non-steady	2.6	11.3	113	0.33	Hatch et al. (1989)

[a]See Appendix A for abbreviations and acronyms. M = mouth exposure by natural breathing; N = nasal exposure by natural breathing; M/N = pooled data from mouth and nasal exposure; Mouthpiece = exposure by mouthpiece; Nasal canula = exposure by nasal canula; Steady = uptake computed during constant unidirectional flow; Non-steady = uptake computed by integration during cyclic breathing; F_t = total RT uptake.
[b]f is either measured or is computed from flows and V_T.
[c]N/A = not applicable.

(equivalent to an inspiratory flow in humans of approximately 1,000 mL/s), the rat data would still be significantly lower than what was measured in humans. The consistency of the human data of Wiester et al. (1996) (in which the same methodology was used to measure F_t in humans as was used for rats) with the other human data strongly suggests that the low F_t in rats is not a function of the methodology employed. Overall, the evidence reasonably points to the conclusion that F_t in a rat is smaller than in a human.

Unidirectional Upper Respiratory Tract Uptake

Table 8-3 summarizes the data on the unidirectional O_3 uptake efficiency of the URT (F_{urt}). In general, F_{urt} describes the uptake efficiency of anatomical structures proximal to the larynx. A possible exception to this are the data of Hu et al. (1992b). In that study, and other bolus studies, it was assumed that the URT is the volume of the RT 50 mL distal to the lips. This may or may not include the larynx.

The wide variety of URT uptake data in different species and under different flow conditions allows some intra- and interspecies comparisons. To make comparisons among different species, however, requires assumptions about the scaling of breathing patterns among species. Gerrity (1989) examined nasopharyngeal uptake data available in different species at that time by examining the data as a function of the ratio of predicted resting flow to measured flow (scaled flow). The predicted flows were obtained using the allometric

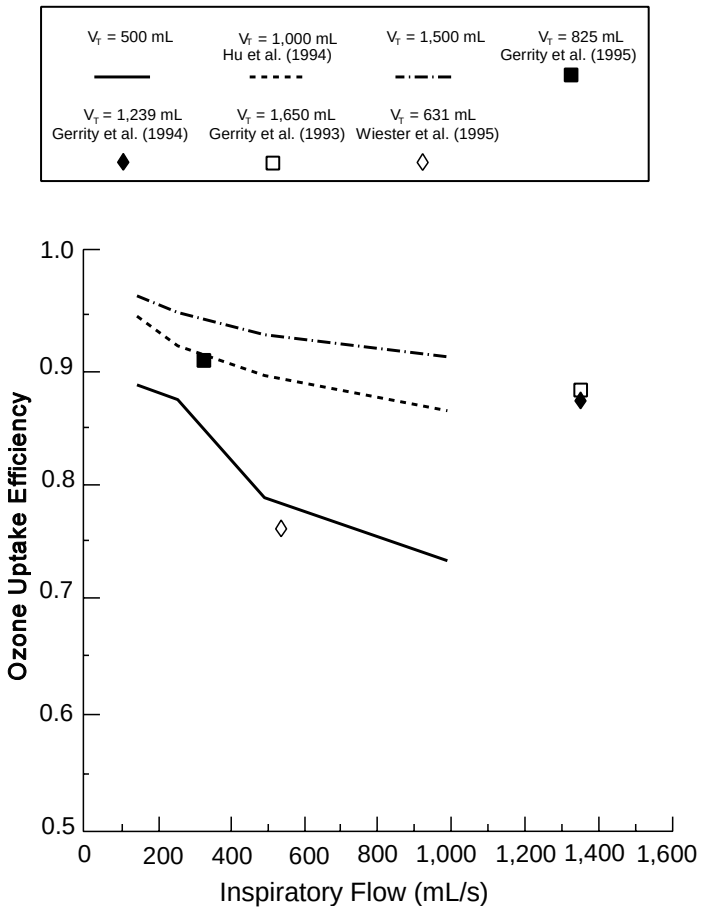


Figure 8-1. Total respiratory tract uptake as a function of inspiratory flow in humans.

equations of Guyton (1947). Ozone uptake efficiency of the URT was then described as a single function of scaled flow and species weight. When newer data become available, this approach can be extended to URT data acquired by both mouth and nasal breathing.

Figure 8-2 shows $1 - F_{urt}$ for nasal breathing plotted as a function of the ratio between predicted and measured flow. As with the previous work of Gerrity (1989), all of the data, except the beagle dog data, are roughly consistent with each other. The body weight dependence of the Gerrity (1989) analysis is illustrated by the two dashed lines representing the predicted range of flow dependencies between rats (lower line) and humans (upper line). All species, except the beagle dogs, fall within these boundaries.

Figure 8-3 shows the data for $1 - F_{urt}$ by the oral route plotted in the same manner. All of the data are for humans except for the two beagle dog data points. Several observations are worth noting. First, the data for the beagle dog appear to be consistent with the human data. The analysis of Gerrity (1989) predicts that the human data generally would lie above the dog data, although not to a large degree. This suggests that the oral passage of the dog may have O_3 scrubbing properties similar to the human oral passage. Second, within the human data, use of a mouthpiece appears to reduce the uptake efficiency of the oral passageway. The closed diamonds in Figure 8-3 are the data from the study of Gerrity et al.

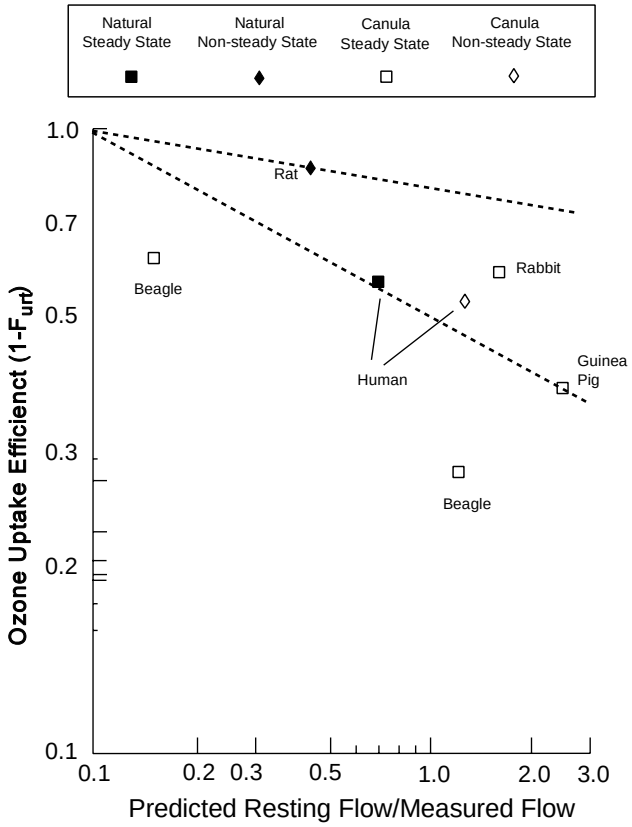


Figure 8-2. Unidirectional uptake efficiency in the upper respiratory tract by the nasal pathway. The ratio of predicted resting flow to measured flow is a way to scale flow to allow for interspecies comparisons. The beagle dog data are from Yokoyama and Frank (1972), the rat data are from Hatch et al. (1989), the rabbit and guinea pig data are from Miller et al. (1979), and the human data are from Gerrity et al. (1988) (closed square) and Ultman et al. (1993) (open diamond). Lines representing predictions for uptake efficiency are from Gerrity (1989).

(1994), which involved unencumbered breathing. These data generally are lower than the data of Hu et al. (1994) and of Gerrity et al. (1995). The fact that the Hu et al. (1994) and Gerrity et al. (1995) data are consistent with each other supports this speculation. This observation may account for the result of Ultman et al. (1993) that the uptake efficiency of the URT is greater by the nasal pathway than by the oral pathway, which is counter to the observations of Gerrity et al. (1988).

Lower Respiratory Tract Uptake

Table 8-4 summarizes the data on the uptake efficiency of the LRT tract (F_{lrt}). In this discussion, F_{lrt} is the uptake efficiency of the LRT relative to the concentration of

O_3 entering the LRT. The human data of Gerrity et al. (1988, 1994, 1995) and the rat data of Hatch et al. (1989) include the larynx in the LRT. The beagle dog data of Yokoyama and

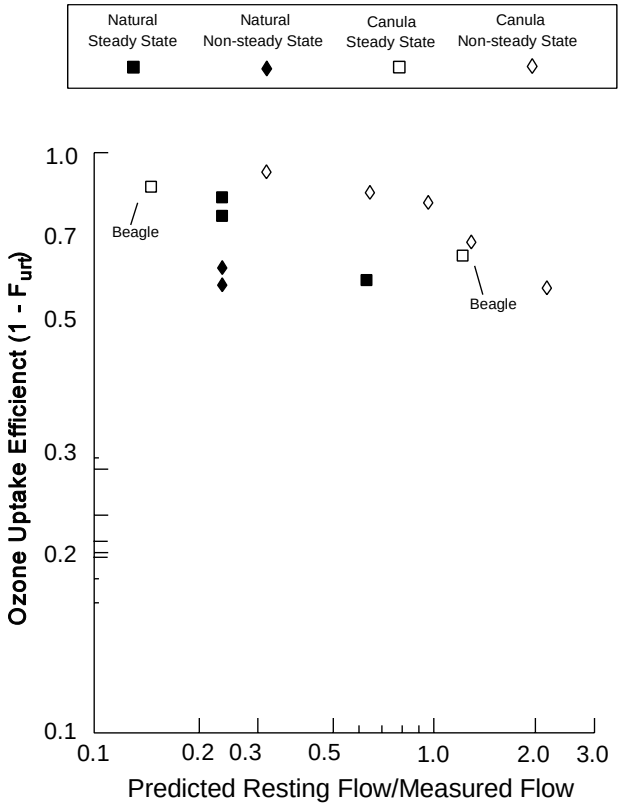


Figure 8-3. Unidirectional uptake efficiency in the upper respiratory tract by the oral pathway. The ratio of predicted resting flow to measured flow scales the flow to allow for interspecies comparison. The closed squares and diamonds at a scaled flow of 0.25 are from Gerrity et al. (1994), the closed square at a scaled flow of 0.65 is from Gerrity et al. (1988), the open diamond at a scaled flow of 0.97 is from Gerrity et al. (1995), the remaining open diamonds are from Hu et al. (1994).

Frank (1972) does not include the larynx. The influence of the larynx in the human data of Hu et al. (1992b) is uncertain because of the volumetric definition of the URT (see discussion above).

Human data obtained using non-steady state methods by the oral pathway are plotted in Figure 8-4 as a function of inspiratory flow. As in Figure 8-1, the data of Hu et al. (1994) are plotted for three different V_Ts: 500 mL, 1,000 mL, and 1,500 mL. The first observation is that the single data point of Gerrity et al. (1995) is approximately 20% higher than the data of Hu et al. (1994) at a comparable flow and V_T. Second, the data of Gerrity et al. (1994) are markedly lower than that suggested for comparable flows and V_Ts by the data of Hu et al. (1994). In assessing this discrepancy, it is important to keep in mind that the URT is defined in the work of Hu et al. (1994) as a fixed volume of 50 mL distal to the lips. This is

an inaccurate definition that could influence greatly the estimation of LRT uptake efficiency. The coherence of all of the data on F_t and F_{urt} by the oral pathway

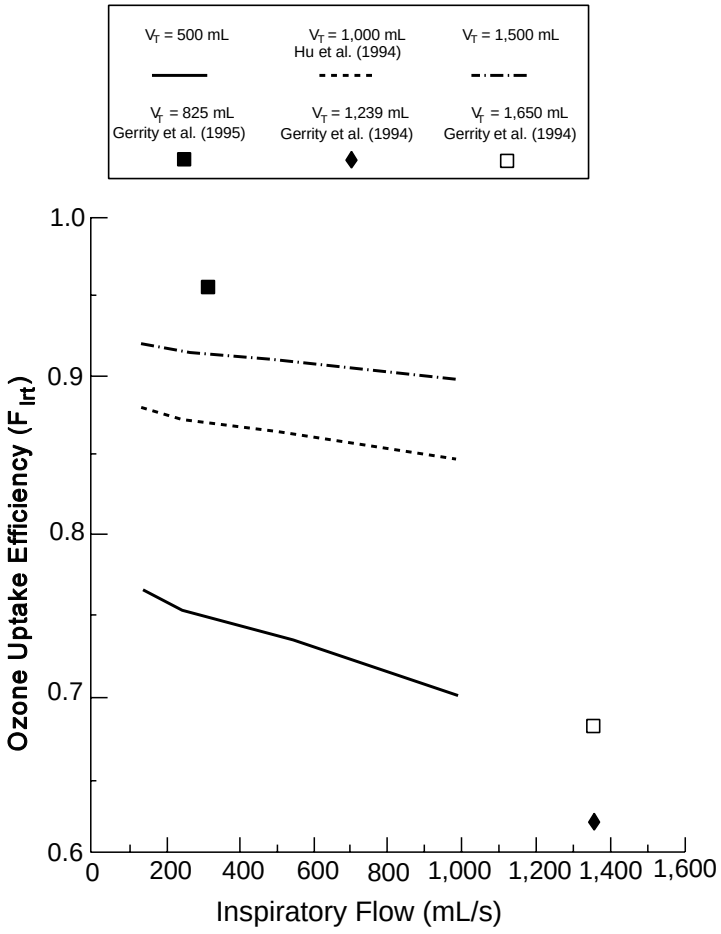


Figure 8-4. Uptake efficiency of the lower respiratory tract as a function of inspiratory flow in humans.

suggests that F_{lrt} also should be consistent among studies. If it is assumed that the data represented by the Gerrity et al. (1994, 1995) studies more accurately reflect human LRT uptake efficiency, then the flow dependence for F_{lrt} would be considerably steeper than suggested by the data of Hu et al. (1994).

Finally, the F_{lrt} data of Yokoyama and Frank (1972) in beagle dogs were acquired with flows that were about 100 and 150% of resting flow rates and V_Ts approximately 150% of those predicted (Guyton, 1947). When flow is scaled in the dogs, the F_{lrt}s of 0.87 and 0.83 at the two flow rates are consistent with the human data of Gerrity et al. (1995) but higher than the Hu et al. (1994) data.

8.2.4 Dosimetry Modeling

8.2.4.1 Background

Table 8-5 presents a summary of theoretical studies of the uptake of O_3 by the RTs (or regions) of humans and laboratory animals that have become available since the 1986 review. Although there are 10 investigations listed, there are only five distinct

Table 8-5. Theoretical Ozone Dosimetry Investigations[a]

Species and Region Modeled/Anatomical Model	Liquid Lining and Tissue Transport and Chemical Reactions	Dosimetry Model /Subject of Investigation[b]	Results/Predictions	Reference
Guinea pig LRT/Kliment (1973), Schreider and Hutchens (1980); rat LRT/ Kliment (1973), Yeh et al. (1979)	Miller et al. (1985)	Enhanced Miller et al. (1985). Investigates the effect on predictions of anatomical models, FRC, ventilation, and TB liquid lining rate constant. Simulates O_3 uptake in anatomical models of rat lobes.	With respect to different anatomical models for the same and different species: qualitative similarity in the shape of net and tissue dose versus airway number curves, but significant differences in regional fractional uptakes. Maximum tissue dose in vicinity of PAR. PAR dose decreases with increasing time of flight to this region. Maximum tissue dose in the vicinity of the first pulmonary region segment of anatomical models.	Overton et al. (1987)
Human, rat, rabbit, and guinea pig LRT/not specified	Miller et al. (1985) and Overton et al. (1987)	Miller et al. (1985) and Overton et al. (1987). Compares O_3 dose profiles of human, rat, guinea pig, and rabbit. Uses model and experimental data to estimate O_3 dose-response curves for decrements in FEV_1 (humans) and for BAL proteins in rat, guinea pig, and rabbit. Compares LRT uptake predictions to the human experimental data of Gerrity et al. (1988).	Similarity among species in the shape of the airway segment curve: tissue dose increases distally in the TB region, reaches a maximum in the first pulmonary region segment for human, rat, and guinea pig and in the last TB segment of the rabbit, and then decreases distally in the pulmonary region. Predictions of uptake distal to the oropharynx are in agreement with Gerrity et al. (1988).	Miller et al. (1988)
Rat total RT/URT: Schreider and Raabe (1981); TB: Uses rat cast data (Raabe et al., 1976) to define TB region paths; PUL: Yeh et al. (1979) for model of the acinus	TB and pulmonary region mass transfer coefficients based on Overton et al. (1987)	Enhanced Overton et al. (1987). Illustrates dose distribution along the longest and shortest (as defined by time of flight) paths from the trachea to the most distal alveoli.	Threefold difference in PAR doses of the shortest and longest paths from trachea to PAR. Dose distributions along the longest or shortest path were qualitatively similar to Overton et al. (1987), with maximum tissue dose in the first pulmonary region generation.	Overton et al. (1989)
Human LRT (newborn to adult)/TB: based on Yeh and Schum (1980); PUL: based on Hansen and Ampaya (1975)	Miller et al. (1985)	Enhanced Overton et al. (1987). From various sources, develops age-dependent LRT anatomical models. For quiet and maximal exercise breathing, applies the dosimetry model of Miller et al. (1985) to several ages from birth to adult, illustrating the LRT distribution of absorbed O_3.	For quiet breathing, the LRT distribution of dose, the percent uptake, and the PAR dose are not very sensitive to age; but are more sensitive during exercise. Regardless of age and breathing state, the largest O_3 dose occurs in the PAR. No uptake in the URT.	Overton and Graham (1989); Miller and Overton (1989)

Table 8-5 (cont'd). Theoretical Ozone Dosimetry Investigations[a]

Species and Region Modeled/Anatomical Model	Liquid Lining and Tissue Transport and Chemical Reactions	Dosimetry Model /Subject of Investigation	Results/Predictions	Reference
Human total RT/URT: Hanna and Scherer (1986); LRT: Weibel (1963)	Time-dependent molecular diffusion and first-order reactions in liquid lining and interstitium; transfer through epithelium modeled as a permeability process—no reactions in this layer. URT and LRT liquid lining rate constants: 50 and 1 times that of Miller et al. (1985), respectively	Model development. Lung dimensions scaled to those of a young male and a young female. Contrasts LRT air-phase concentrations during exercise and rest. Compares male and female air-phase O_3 concentrations and male and female subepithelial concentrations.	URT uptake may be greater in cold than in warmer air. For the ventilatory parameters used, (1) subepithelial O_3 concentrations are a maximum in the terminal bronchioles or the first respiratory bronchioles, and (2) these concentrations are greater in the female than in the male for most of the RT.	Hanna et al. (1989)
Human, distal segment of a lobe/ based on Horsfield et al. (1971)	Similar to Miller et al. (1985)	Monte Carlo simulation; transport processes defined in terms of probabilities based on physical and chemical principles. The effect of lung asymmetries on the distribution of uptake in the pulmonary region.	Tissue dose in the PAR along the shortest path is approximately 50% larger than that along the longest path.	Ultman and Anjilvel (1990)
Human LRT/Weibel (1963)	Investigates two formulations: (1) Miller et al. (1978) and (2) Miller et al. (1985)	Model development; assumes quasi-steady conditions. Air-phase concentration and tissue dose profiles for the two reaction schemes, various ventilatory parameters, and various liquid lining transport and chemical parameters. Compares predictions of first-order reaction scheme to results of Miller et al. (1985).	This model does not conserve mass. Predictions should only be considered qualitatively. Maximum tissue dose in respiratory bronchioles for both chemical reaction schemes.	Grotberg et al. (1990) (Grotberg [1990])
Rat LRT/based on serial reconstruction of a set of intrapulmonary airways and their ventilatory units combined with a single path from the larynx to the reconstructed set based on Yeh et al. (1979)	TB and pulmonary region mass transfer coefficients based Overton et al. (1987)	Model development. Illustrates the influence of ventilatory unit size and proximal anatomic dead space and on the uptake and distribution of inhaled O_3 in ventilatory units. (Illustrates the influence of ventilatory unit volume on the distribution of inhaled O_3 within ventilatory units.)	Ventilatory unit uptake is significantly influenced by both proximal airway dead space and ventilatory unit volume. Flux of O_3 to air-liquid interface in the proximal portions of larger ventilatory units are significantly greater than in smaller units.	Mercer et al. (1991) (Mercer and Crapo [1993])

Table 8-5 (cont'd). Theoretical Ozone Dosimetry Investigations[a]

Species and Region Modeled/Anatomical Model	Liquid Lining and Tissue Transport and Chemical Reactions	Dosimetry Model /Subject of Investigation[b]	Results/Predictions	Reference
Rat ventilatory unit/ Mercer et al. (1991)	Mercer et al. (1991)	Mercer et al. (1991). Along a path distally from a bronchiolar-alveolar duct junction; compares experimentally determined changes in ventilatory unit wall thickness due to an O_3 exposure to dosimetry model predictions of flux to the air-liquid interface.	As a function of distance from the BADJ; experimentally determined relative changes in ventilatory unit wall thickness due to O_3 exposure are very similar to predicted relative fluxes to the air-liquid interface.	Pinkerton et al. (1992); Miller and Conolly (1995)
Human total RT/URT: Fredberg et al. (1980); LRT: Weibel (1963)	Pseudo steady-state diffusion and first order reactions combined with biochemical data of Miller et al. (1985)	Model development. Illustrates LRT distribution of (1) air-phase concentration at various times during the breathing cycle and (2) O_3 flux (dose) to liquid lining and to tissue.	Flux of O_3 to air-liquid interface decreases distally; flux to tissue increases along the conducting airways, reaches a maximum in the terminal bronchioles, then decreases rapidly in the gas exchange region.	Hu et al. (1992a)

[a]See Appendix A for abbreviations and acronyms. Generally, for modeling purposes, PAR is the first pulmonary region, generation, or model segment; PUL = pulmonary region.
[b]Refers to the theoretical or mathematical formulation aspects of gas transport and reactions without the specification of morphometric and physiological parameter values.

dosimetry models (with respect to groups of co-workers and independent model formulation): the models of (1) Overton et al. (1987) and Miller et al. (1985); (2) Hanna et al. (1989); (3) Grotberg et al. (1990), although they considered two reaction schemes; (4) Mercer et al. (1991); and (5) Hu et al. (1992a). In some cases, several references have been grouped into one investigation. This is because the multiple studies came from the same co-workers or laboratory and added to or were complementary to the original or common dosimetry modeling theme.

Major factors affecting the local uptake of reactive gases in the RT were respiratory tract morphology and anatomy; the route of breathing (nose or mouth); the depth and rate of breathing (V_T and f); the physicochemical properties of the gases; the processes of gas transport; and the physicochemical properties of the liquid lining of the RT, respiratory tract tissue, and capillary blood. A detailed discussion of these factors can be found in Overton (1984), Ultman (1988), and Overton and Miller (1988).

Because all of the dosimetry models listed in Table 8-5 were developed to simulate uptake in the LRT or the total RT, these models have some common aspects. These include the formulation of O_3 transport and wall loss in the air compartments of the RT, the use of species-dependent morphometric models or data to define air and liquid lining compartment dimensions, and a description of the transport and loss of O_3 in the liquid lining and tissue.

In all the dosimetry models that have become available since 1986, except for Ultman and Anjilvel (1990) and Grotberg et al. (1990), which are discussed later, O_3 transport and loss processes in air compartments were approximated in terms of a one-dimensional, time-dependent, partial differential equation of continuity. This type of equation accounts for axial convection and dispersion or diffusion and the loss of O_3 by absorption at the gas-liquid interface. The use of this approximation is very common in modeling the transport in the LRT of gases such as oxygen, nitrogen, helium, and CO_2 (e.g., Scherer et al., 1972; Paiva, 1973; Chang and Farhi, 1973; Yu, 1975; Pack et al., 1977) and has been assumed to be applicable to O_3. Ultman and Anjilvel (1990) used a Monte Carlo method to simulate O_3 uptake. Based on the physical and chemical principles of mass transport in the RT, probabilities were assigned to the fate of a molecule in a way so as to account for convection, dispersion, and loss to the liquid lining.

Dosimetry models published since 1986 can be grouped according to how transport and chemical reactions are modeled in respiratory tract fluids and tissues: those based on the formulation of (1) Miller (1977) and Miller et al. (1978), who used an instantaneous reaction scheme, and (2) Miller et al. (1985), who used a quasi-steady, first-order reaction scheme. These two approaches are discussed in the earlier criteria document (U.S. Environmental Protection Agency, 1986). In addition to the use of similar formulations for liquid and tissue transport/reactions, all of the post-1986 studies used essentially the same biochemical data of either Miller et al. (1985) for humans or Overton et al. (1987) for laboratory animals. The implication is that most of the studies are expected to predict qualitatively similar results.

There are minor variations on the second chemical reaction formulation. Hanna et al. (1989) used a time-dependent diffusion-reaction equation, instead of the time-independent (quasi-steady) equation used by Miller et al. (1985). Based on the rate constants used by Hanna et al. (1989) and on discussions in Miller et al. (1985) and in Grotberg et al. (1990), the modeled transport processes in the liquids and tissues can be inferred as essentially quasi-steady, which is equivalent to the second formulation. Another variation uses mass transfer coefficients determined by the second formulation and the biochemical assumptions of Miller

et al. (1985) or Overton et al. (1987). The liquid and tissue transport/reaction formulation for specific investigations is indicated in column 3 of Table 8-5.

In addition to the assumptions and the formulation of equations that describe the transport and loss of O_3 in the RTs of humans or laboratory animals, it is important to evaluate whether simulation results reflect accurate solutions to the mathematical dosimetry model formulation. Of the five distinct model formulations listed in Table 8-5, Overton et al. (1987), Mercer et al. (1991), and Hu et al. (1992a) discuss most or all of the relevant issues of stability, solution convergence, and mass conservation. In addition, using steady unidirectional flow in a straight tube as a test case, they report successfully simulating analytical solutions to their equations of transport and uptake. Neither Hanna et al. (1989), Ultman and Anjilvel (1990), nor Grotberg et al. (1990) address the issue of accuracy. There is no reasonable way to judge whether the solutions of Hanna et al. (1989) or of Ultman and Anjilvel (1990) accurately represent solutions to their dosimetry model assumptions and formulations; however, with the exception of Grotberg et al. (1990), there are no reasons to assume that the solutions of these models are not accurate.

Because the Grotberg et al. (1990) model formulation is different than the others, an explanation is needed. Based on the smallness of relevant parameters, Grotberg et al. (1990) assume quasi-steady conditions for O_3 concentration and air velocity in the air compartments and obtain approximate analytical solutions to the time-independent, three-dimensional equation of continuity for a model airway and apply the results to the morphometric model of Weibel (1963). One advantage of analytical solutions is that they account naturally for parameters (such as dispersion and gas-phase, mass-transfer coefficients) or local processes (e.g., possibility of high uptake at airway entrances) that must be known and estimated for, or incorporated into, the one-dimensional approach. Grotberg et al. (1990) carried out simulations using anatomical and physiological conditions based on Miller et al. (1985) and compared their results. Although qualitatively similar to Miller et al. (1985), Grotberg and co-workers predicted significantly larger pulmonary tissue doses (up to 10-fold).
A comparison of the pulmonary region doses predicted by Grotberg et al. (1990) to those predicted by Miller et al. (1985) indicates that the Grotberg et al. (1990) model does not conserve mass (it predicts that the pulmonary region absorbs over 3.4 times the amount of O_3 inhaled). The overprediction may be an artifact of the quasi-steady approximation, because effects due to differences in the time of flights from the trachea to different LRT locations are not taken into account. In any case, the quantitative predictions reported by Grotberg et al. (1990) are questionable.

Chemical data more recent than that used for the dosimetry models in Table 8-5 show that compounds other than unsaturated fatty acids (the only compound with which O_3 is assumed to react in the models using the second chemical reaction formulation) are as reactive or more reactive with O_3 (Pryor, 1992). Using these data, estimates of O_3 diffusion coefficients in the liquid lining and bilayers, layer thicknesses, and data on the concentrations of biocompounds in these layers, Pryor (1992) estimates that most of any O_3 that penetrates into a cell bilayer reacts within the layer (very little if any penetrates to the cell interior), and O_3 will not penetrate the liquid lining where it is greater than 0.1 μm thick. Several relevant comments are given by Pryor (1992): the calculations are considered a crude first approximation; the possibility that a small fraction of O_3 may penetrate the bilayer and reach the cell interior can not be excluded; and surfactant layers can be very thin, and some cells may not be protected very well or at all.

If the conclusions of Pryor (1992) are essentially correct, they have implications for past and future dosimetry modeling studies because past investigations have underestimated the reactivity of O_3 with biocompounds; with respect to cellular damage, products of O_3 reactions in the liquid lining may be the main toxic compound; increasing the value of rate constants would have no effect on predictions of dosimetry models using the instantaneous reaction scheme because rate constants in this approach are assumed to be infinite, but increasing the concentration of reacting biocompounds would increase uptake; (4) use of unsaturated fatty acid data only (with a first-order reaction scheme) results in an underestimate of the reactivity of O_3 in the liquid lining and an overestimate of the O_3 tissue dose, and a possible underestimation of the toxic dose due to reaction products; and (5) with higher O_3 reaction rates, the first-order chemical-reaction formulation would result in larger predicted uptakes.

8.2.4.2 Dosimetry Model Predictions

Similarity of Model Predictions

A survey of dosimetry modeling results shows that, in some areas of investigation, there is a qualitative similarity in predictions by models of different groups of investigators for different species or subpopulations.

(1) *Distribution of LRT dose (dose profiles or dose versus generation).* As shown in Figure 8-5, beginning at the trachea, net dose (O_3 flux to air-liquid interface) slowly decreases distally in the tracheobronchial region (TB) and rapidly decreases distally in the pulmonary region. Tissue dose (O_3 flux to liquid-tissue interface) is very low in the trachea, increases to a maximum in the terminal bronchioles or first airway generation in the pulmonary region, and rapidly decreases distally from this location (e.g., Miller et al., 1978, 1985, 1988; Overton et al., 1987, 1989; Overton and Graham, 1989; Grotberg et al., 1990; Hu et al., 1992a).

If O_3 were the only toxic agent and all the tissues of the LRT were equally sensitive to the same dose, the models predict that the greatest morphological damage would occur in the vicinity of the junction of the conducting airways and the pulmonary region and decrease rapidly (distally) from this area, which is consistent with observations in laboratory animals (see Chapter 6, Section 6.2.4). On the other hand, using the best estimates of morphometric and physiologically based biochemical parameters of Miller et al. (1978, 1985) and Overton et al. (1987), the models predict extremely (relatively) low tissue doses in the trachea and large bronchi; this suggests very little or no tissue damage should occur there, which is contrary to observations (see Chapter 6, Section 6.2.4). However, this is moot, if, as suggested by Pryor (1992), the toxic substances are primarily reaction products of O_3 and not O_3 itself. In this case, the O_3 net local dose, not the local O_3 tissue dose, may be a better estimator of local toxic tissue dose, because the rate of production of products would be related to the rate of O_3 uptake.

(2) *Effect of exercise or increased ventilation.* The effect of exercise is to slightly increase the TB dose and to significantly increase the pulmonary region total dose (mass of O_3) and the CAR dose (mass per unit surface area) (e.g., Miller et al., 1979, 1985; Overton et al., 1987, 1989; Overton and Graham, 1989; Hanna et al., 1989; Grotberg et al., 1990).

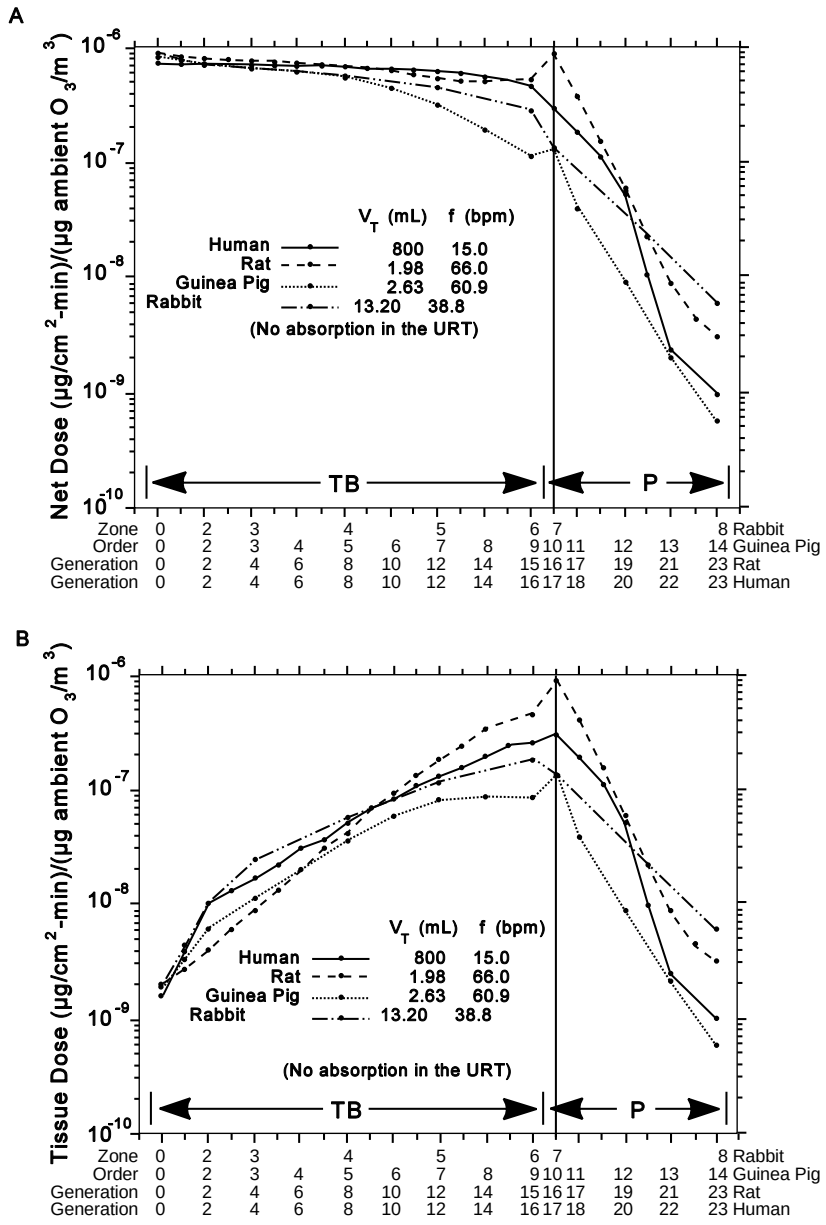


Figure 8-5. Net dose (A) and tissue dose (B) versus sequential segments along anatomical model airway paths for human, rat, guinea pig, and rabbit. In general, each segment represents a group of airways or ducts, with common features as defined by the designers of the anatomical model (human and rat: generation; guinea pig: order; rabbit: zone). For a given species, the plotted dots represent a predicted dose that corresponds to a given segment. The dots have been joined by lines for ease of interpreting the plots; these lines do not represent predicted values except where they intercept the dots. TB = tracheobronchial region; P = pulmonary region.

(3) *Effect of respiratory tract inhomogeneity*. Models have predicted that the further the proximal alveolar region is from the trachea, the less the O_3 tissue dose (mass of O_3 absorbed per unit surface area) to the proximal alveolar region. (For modeling purposes, the proximal alveolar region has been defined as the first pulmonary generation or the first pulmonary region model segment along a path; this region is a part of the CAR.) Overton et al. (1989) predicted a threefold greater proximal alveolar region dose for the shortest path relative to the longest path in rats. Ultman and Anjilvel (1990) simulated O_3 distribution in a small segment ($<1\%$) of the distal airways of an asymmetric anatomic model of the human lung. They found that the O_3 tissue dose (mass per square centimeter) in the proximal alveolar region along the shortest path was approximately 50% greater than that along the longest path. Mercer et al. (1991) found that path distance and ventilatory unit size affect dose: predicted doses in the proximal segments (essentially, the proximal alveolar region) of the larger ventilatory units (with the smallest relative dead space) are significantly larger than the average proximal segment doses. Further, for the small sample of ventilatory units modeled (43), Mercer et al. (1991) predicted a range of proximal segment doses of greater than a factor of 6. Because the proximal alveolar regions of Ultman and Anjilvel (1990) and of Mercer et al. (1991) belonged to a "local cluster", and there are many clusters with varying distances from the trachea, a variability greater than 50% and a factor greater than 6, respectively, are expected in proximal alveolar region doses. Mercer and Crapo (1993) illustrated the effect of ventilatory unit volume alone on the distribution of dose, predicting that a 2.3 times larger unit receives 1.9 times the dose (mass per surface area) of the smaller unit at the entrance of the unit.

The variability of predicted proximal alveolar region doses and, by inference, CAR doses suggests that the magnitude of toxicological effects for different CARs are different. This prediction is consistent with the observations of Schwartz et al. (1976) and Boorman et al. (1980) of damage variation among different CARs of the same rat. It is reasonable to assume that variable damage at equivalent but different morphological locations also occurs in humans.

Specific Topics

Effect of Assumptions About Anatomical Dimensions. For rats and guinea pigs, Overton et al. (1987) used two morphometrically based anatomical models (rat anatomical models: Kliment, 1973, and Yeh et al., 1979; guinea pig anatomical models: Kliment, 1973, and Schreider and Hutchens, 1980) to investigate the influence of anatomical model formulation on predicted uptake. Results with all four anatomical models in combination with different ventilatory parameters showed a qualitative similarity in the shapes of the dose profiles, but the two anatomical models for the same species resulted in considerable differences in predicted percent RT and pulmonary region uptakes.

Respiratory Tract Uptake in Human Adults and Children. Overton and Graham (1989) used several sources of data on age-dependent LRT dimensions and structure to construct theoretical LRT anatomical models for humans from birth to adulthood. The O_3 dosimetry model of Miller et al. (1985) was used to estimate the regional and local uptake of O_3. For the percent uptake (84 to 88%) during quiet breathing, the LRT distribution of

absorbed O_3 and the centriacinar O_3 tissue dose are not very sensitive to age. Regional percent uptakes are more dependent on age during heavy exercise or work than during quiet breathing, and total uptakes range from 87 to 93%. Generally, the total quantity of O_3 absorbed per minute increases with age. For all conditions simulated, the largest O_3 tissue dose is predicted to occur in the CAR. Miller and Overton (1989) present similar results. Because uptake by the URT was not simulated and because this region can be assumed to have an important effect on LRT uptake, a comparison of predictions of LRT uptakes in children and adults should be viewed with caution. On the other hand, URT uptake probably has little effect on the shape of the dose curves.

Dosimetry Modeling Results Compared to Dosimetry Data

Based on the experimental conditions discussed in Gerrity et al. (1988) and using the model and parameters of Miller et al. (1985), Miller et al. (1988) simulated the uptake of O_3 distal to the oropharynx of human subjects. For the target f's of 12 and 24 bpm and V_Ts ranging from 0.4 to >1.6 L, the simulation results were in good agreement with the breath-by-breath experimental data. The average experimental LRT uptake efficiency was 0.91 as compared to the 0.89 prediction given by Miller et al. (1985) for the region distal to the oropharynx. It should be remembered, however, that values for uptake efficiency from the Gerrity et al. (1988) study were derived from the raw data using a steady-state method, whereas the models of Miller et al. (1985) and Miller et al. (1988) utilize cyclic flow, thus making the predictions more appropriate for comparison with uptake data from non-steady state methods. From an analysis in Gerrity et al. (1994), it appears that total RT uptake computed by either steady-state or non-steady state methods differ by only about 10% in relative terms.

There have been major improvements to the original model, as described by Miller et al. (1985) and Overton et al. (1987), including the addition of the URT and establishing a regional mass transfer coefficient based on experimental data. Table 8-6 summarizes the assumptions that underpin these improvements, as well as other relevant assumptions used for simulating the uptake of several human dosimetry experiments. A discussion of the assumptions is given in Section 8.5.2.

After taking into consideration the assumptions of Table 8-6, Table 8-7 compares experimental total RT uptake efficiency data and model predictions for humans. Use of the rat assumptions in conjunction with the model will be discussed later (Section 8.4.3). The model predictions show good agreement with the total RT uptake efficiency data of Gerrity et al. (1988), Gerrity et al. (1994), and Hu et al. (1992b). In all cases, the predictions are within 10% of the measured values. The agreement with the data of Hu et al. (1992b) is even better, as expected.

The model prediction for the data of Wiester et al. (1996) is less accurate. Comparison of the Wiester et al. (1996) data with the Hu et al. (1994) data (Figure 8-1) shows, however, that the results are in good agreement with each other. Thus, it would appear that the V_T dependence of the model does not necessarily reflect the real world. However, the general agreement between the model predictions and data are quite good.

Although the models are capable of making reasonable predictions of total RT uptake efficiency, their accuracy for specific regions remains uncertain. The O_3 bolus data (Hu et al., 1992b, 1994; Ultman et al., 1993) and the airway uptake efficiency data

Table 8-6. Assumption for Application of Dosimetry Model to Breathing Frequency Responses to Ozone[a]

Species	Mode of Breathing	Respiratory Tract Morphology	Mass Transfer Coefficients
Human	Oral	- LRT structure from Weibel (1963). - Volume of oral cavity through larynx and surface to volume ratio (S/V) from Hu et al. (1992a,b). - Dead space volume (V_d) and FRC from Hart et al. (1963); TB region volume at FRC equals V_d minus oropharyngeal volume. - Proximal alveolar region defined as first respiratory bronchiole. - Pulmonary region expands; TB does not expand.	- Mass transfer coefficients for each oropharyngeal segment and each TB generation defined as Ka/(S/V) where S/V for the TB region is from Weibel (1963) dimensions reduced to FRC; Ka from Hu et al. (1992b). - Pulmonary mass transfer coefficient is 0.10 cm/s (Miller et al., 1985).
Rat	Nasal	- NP dimensions from Schreider and Raabe (1981). - TB region from Yeh et al. (1979). - Volumes and surface areas of LRT isotropically scaled to FRC. - Pulmonary region from Mercer et al. (1991). - TB and pulmonary regions expand uniformly during breathing. - Proximal alveolar region is first generation of pulmonary region.	- NP and TB mass transfer coefficients estimated using data of Hatch et al. (1989). - Mass transfer coefficient of pulmonary region = 0.137 cm/s; inferred from Pinkerton et al. (1992).

[a]See Appendix A for abbreviations and acronyms.

Table 8-7. Comparison of Total Respiratory Tract Uptake Data with Model Predictions[a]

V_T (mL)	f (bpm)	Measured F_t	Predicted F_t	Data Source
832	12	0.97	0.96	Gerrity et al. (1988)
832	24	0.96	0.93	Gerrity et al. (1988)
500	15	0.86	0.89	Hu et al. (1992b)
1,000	7.5	0.93	0.94	Hu et al. (1992b)
1,650	25	0.88	0.95	Gerrity et al. (1994)
1,239	35	0.87	0.93	Gerrity et al. (1994)
631	16	0.76	0.94	Wiester et al. (1996)

[a]See Appendix A for abbreviations and acronyms.

(Gerrity et al., 1995) raise questions about the model predictions of uptake in the conducting airways. These latter data sets suggest that the models of Miller et al. (1985, 1988) may underestimate the O_3 uptake coefficients in the conducting airways. Ultman et al. (1993) show in their analysis of the bolus data that the reactivity of O_3 with the lung liquid lining decreases with increasing depth into the lung. This could imply that more O_3 is taken up in central airways than previously had been thought. However, the predictions presented in Table 8-7, which are based on the assumptions of Table 8-6, represent a revision of the Miller et al. (1985) model in that the mass transfer coefficients are derived from the actual human data of Hu et al. (1992b).

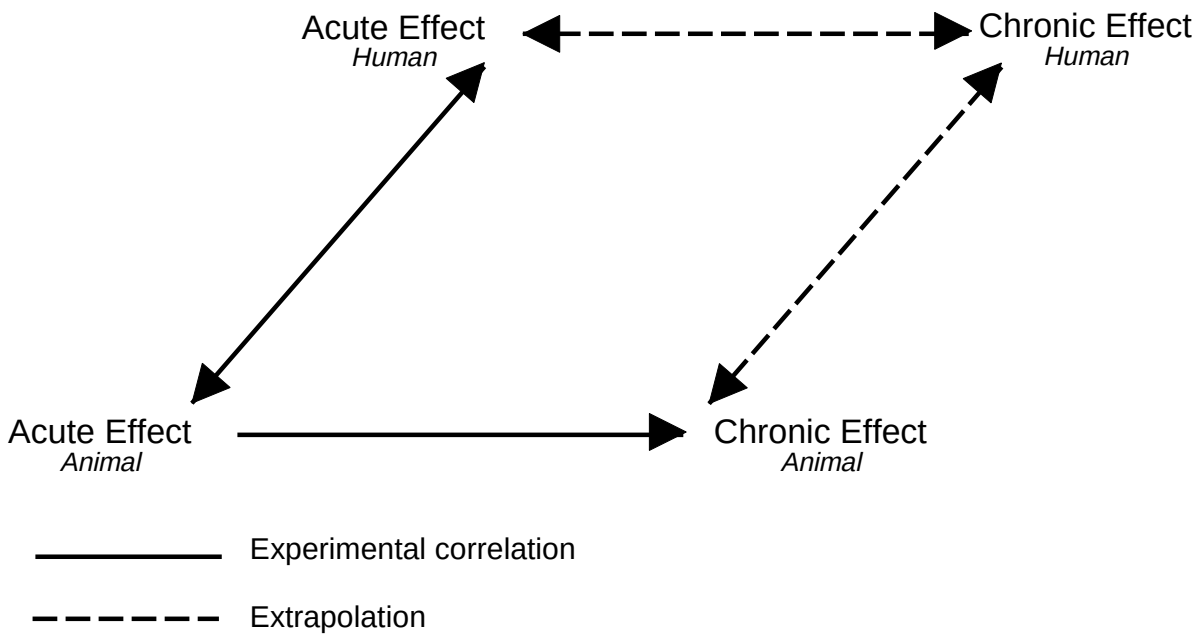
Because the utility of dosimetry models is their usefuleness in facilitating interspecies extrapolation, it is important to compare predictions with animal data as well as with human data. Using the Overton et al. (1987) model formulation and parameters, Overton et al. (1989) developed a formula that can be used to calculate LRT uptakes in rats, given their V_Ts and f's. For comparison purposes, the data of Wiester et al. (1987, 1988) and Hatch et al. (1989) can be used. The average uptake efficiency for the rat from these data is 0.45. Based on the V_Ts and f's of these animals, an average LRT uptake of 0.61 is computed using Overton et al. (1989). If Overton et al. (1989) had included the effects of URT uptake, their model would have predicted more than 0.61.

It is important to note that these results are based on the older model assumptions, not those presented in Table 8-6. If the newer assumptions had been used, the agreement between predictions and actual results would have been much better (i.e., total uptake would have been in the 50 to 60% range, depending on ventilation).

8.3 Species Sensitivity: Lung Function and Inflammatory Endpoints Exemplifying an Approach

8.3.1 Introduction

Quantitative extrapolation of animal-based O_3 toxicity data to the human circumstance requires a paradigm that includes both an estimate of target tissue dose (dosimetry) and an algorithm that relates the responsiveness of the test species to that of the human (species sensitivity). This paradigm can be depicted as an extrapolation parallelogram (Figure 8-6), which conceptualizes a relationship between chronic animal study data and long-term human health effects based on an understanding of acute effects in both species (Graham and Hatch, 1984). Although recent studies have begun to elucidate the underlying mechanisms determining response, the bulk of the present O_3 toxicity database in animals and humans remains largely descriptive. Hence, only a simplified application of this paradigm is feasible at this time. The following section will attempt to harmonize selective literature on acute human and animal responses to O_3 exposure (already reviewed in detail in Chapters 6 and 7) with what is known about the dosimetry of O_3, in an effort to discern relative species sensitivity. To construct an argument that is plausible for this test application, focus is on endpoints for which there are sufficient data in both humans and test animal species and for which exposure scenarios are similar. The endpoints compared include measures of pulmonary function and markers of lung inflammation, most notably BAL protein and cells. When possible, other influencing parameters, such as ventilation augmentation and antioxidants within the lung also will be discussed. The body of in vitro cell studies has not been included because of the



difficulties in interpretation associated with ***Figure 8-6.***

Parallelogram paradigm for utilizing animal data for human health predictions. Acute homologous endpoints serve as the basis for extrapolating chronic effects in humans from animal data.

dosimetry and culturing systems. The reader is referred to Chapter 7, Section 7.2.5, and the recent review by Koren et al. (1994). The goal here is to develop a hypothetical model for the assessment of species-specific sensitivity with acute O_3 exposure that can serve as a framework to better predict human responses, especially with regard to chronic effects. The complex issue of whether controlled human clinical studies accurately reflect population-based responses also will not be considered in this discussion.

8.3.1.1 Dosimetry

This topic has been discussed in detail in Section 8.2. Recent studies by Hatch et al. (1994), utilizing the nonradioactive isotope of oxygen, ^{18}O, to label O_3, have shown that exposure of exercising humans (60 L/min) and resting rats to 0.4 ppm O_3 for 2 h resulted in 4 or 5 times the ^{18}O dose (as adduct) to the BAL constituents of humans as compared to those of F344 male rats. This four- to fivefold difference appeared to be due to the exercise-stimulated hyperventilation of the humans when compared to the rat and compared favorably with indices of effect (i.e., BAL cells and protein at 24 h). Only when the rats were exposed to 2 ppm O_3 for 2 h did the $^{18}O_3$ labeling of BAL constituents approximate that of the human. Thus, on the basis of this study of cellular and protein influx due to O_3 injury, the rat and human appear to have similar sensitivity to O_3 when exercise is considered. Additional related studies with $^{18}O_3$ indicate that deposition in the RT is a cumulative function of ventilation over the initial period of exposure, which would lend support to these findings (Santrock et al.,

1989). Attempts to compare animal data obtained without exercise to human study data with exercise thus would underestimate the dose to the lung and presumably the resultant risk of effect. Studies of O_3 effects (but without assessment of dose) in exercising rodents have confirmed this conclusion (Mautz et al., 1985).

Exercise is only one factor that can alter dose and effect. Studies in laboratory animals that incorporate other factors, such as time of day or diurnal rhythms (Van Bree et al., 1992), animal strain (Pino et al., 1991; Costa et al., 1993), or nutrition (Chapter 6, Section 6.2.5), also show substantial modification of response to O_3, and thus emphasize the need for careful consideration of exogenous factors when attempting to compare or extrapolate study findings. It is likely that a similar range of factor-dependent variability exists within human test subjects.

8.3.2 Homology of Response

The concept of species sensitivity actually consists of two integrated components. The first, homology of response, indicates whether the outcome seen in the animal test species represents the same biological response in the human. In many cases, a measurement of the same endpoint in both species can be presumed to reflect the same toxic phenomenon or mechanism (i.e., the pulmonary irritant-induced tachypnea) (see below). On the other hand, there may be endpoints that, although homologous, are not expressed similarly; for example, the burning discomfort of sensory irritation in the human and the pause on tidal expiration seen in rodents. The second component of species sensitivity relates the dose-response curve to given homologous responses. Alterations in permeability of the air-blood barrier of the lung appear to reflect true species differences in sensitivity to pulmonary irritants such as O_3 (Hatch et al., 1986). Ideally, these elements of species sensitivity should flow directly into extrapolation formulae developed to integrate animal and human research data.

8.3.2.1 Lung Function Endpoints as Homologous Indicators

Lung function studies of small mammals have provided basic physiological information important to the understanding of both normal and diseased lungs (Snider and Sherter, 1977; Harkema et al., 1982; Raub et al., 1982; Mauderly, 1984; Costa, 1985). Animal lung function tests, adapted from those used clinically, have proven useful in describing the nature and severity of lung injury and in distinguishing toxicant-induced effects in the central or peripheral airways from those effects in the parenchyma. In practice, the interpretation of functional changes detected in animals derives from knowledge and experience in human pulmonary medicine. Supporting this view, in theory, is the allometric database for normal mammals, in which the lung function variables associated with ventilation and aerobic metabolism scale systematically to body mass over nearly seven orders of magnitude (Stahl, 1967; Leith, 1976). The lung function studies of O_3 toxicity in animals and humans considered in the present discussion are described in detail in Chapters 6 and 7, Sections 6.2.5 and 7.2, respectively, of this document and in the previous O_3 criteria document (U.S. Environmental Protection Agency, 1986).

8.3.2.2 Inflammatory and Antioxidant Endpoints as Homologous Indicators

Inflammation of pulmonary airways and airspaces is best described as a cascade of events that network infiltrating leukocytes, plasma proteins, and cell-derived mediators, which function presumably to defend or repair (but may further damage) the injured lung (see

Chapters 6 and 7, Sections 6.2.2 and 7.2.4, respectively; Koren et al., 1994). Key markers of the basic inflammatory process include plasma-derived proteins, such as albumin, globulins, and plasmin, and a primary inflammatory cell, the polymorphonuclear leukocyte (PMN). Of these two markers, plasma-derived proteins in the acute phase generally are thought to represent a "leak" from the vasculature to the airspace lumen. Hence, under controlled temporal conditions, plasma protein residing in the airspace and accessible by BAL can be used as a proportional marker of effect that, in turn, should be related to dose. The presence of PMNs in the airspaces is a bit more complex because of the signals involved in recruiting these cells into the lung lumen after injury and the cascade of events apparently involved in their poiesis from the vasculature to the lung lumen. For the purposes of species comparison, plasma-derived protein (nonspecific) and the proportion of PMNs among total cells as sampled by BAL will be emphasized as primary indices of damage and inflammation within the lung.

Antioxidant substances in lung tissue (Slade et al., 1985) and BAL fluid and cells (Slade et al., 1993) have been identified and quantified for humans and several laboratory animal species. The species profiles of these antioxidants in the lung tissue and their respective BAL cells and fluid can differ appreciably (Table 8-8), but collectively they appear to play a significant role in defense of the lung against both endogenous and exogenous oxidant challenge. In particular, ascorbate and vitamin E appear to have major functions in protecting the lung from O_3 challenge (Chapter 6, Section 6.2.1; Slade et al., 1989; Crissman et al., 1993; Koren et al., 1989b; Elsayed et al., 1988), and, when their levels are manipulated in vivo, either can influence the degree of toxic outcome. Hence, the measurement of basal and O_3 response levels of these antioxidants in BAL cells and fluids is useful in assessing the qualitative and quantitative responses among humans and laboratory test species.

8.3.3 Studies of Lung Function

8.3.3.1 Confounding Influences in Lung Function Studies

Ideally, a system for measuring pulmonary function in small animals would be approximately the same as that used in humans for cooperative, unrestrained subjects. However, in animal studies, this is usually not possible. Fortunately, certain measures (e.g., static lung volumes, diffusion capacity) appear to be minimally influenced by sensory reflex or muscular activity in spite of unnatural stresses or blunting of responses caused by anesthetic or physical immobilization. On the other hand, some measurements, typically those involved in the assessment of ventilatory mechanics, can be profoundly influenced by these and other factors, such as ambient and toxicant-altered body core temperature, thus confounding cross-species comparisons. Because a major emphasis of this section is the comparison of lung function data of animals and humans, it is important that the reader realize potentially confounding influences borne by studies of lung function in rodents when compared to analogous measurements in humans. These are discussed briefly below.

Anesthesia

Anesthesia alters pulmonary function measurements in both humans (Rehder et al., 1975) and laboratory animals (Skornick and Brain, 1990; Lamm et al., 1982; Rich et al.,

Table 8-8. Pulmonary Antioxidant Substances in Various Laboratory Animal Species and Humans[a]

Antioxidant	Mouse	Hamster	Rat	Guinea Pig	Rabbit	Human
Ascorbate						
Tissue[b]	41±4	26±2	34±2	39±1	27±3	22±7
BAL cells[c]	—	—	50.3±5.4	17.9±1.4	—	3.5±0.1
BAL fluid[c]	—	—	199.4±9.1	28.8±2.2	—	21.4±2.8
Glutathione						
Tissue[b]	62±3	61±2	50±2	83±3	83±3	7±1
BAL cells[c]	—	—	14.8±2.7	14.6±2.4	—	2.9±0.5
BAL fluid[c]	—	—	12.1±5.0	11.2±1.9	—	20.4±3.8
Tocopherol						
Tissue[b]	1.0±0.1	1.0±0.1	2.1±0.1	2.0±0.2	1.4±0.2	0.8±0.1
BAL cells[d]	—	—	577.7±83.1	454.5±58.2	—	95.1±23.4
BAL fluid[d]	—	—	0.6±0.2	1.4±0.5	—	47.2±3.8
Uric Acid						
Tissue[b]	—	—	0.35±0.05	4.14±0.24	—	—
BAL cells[c]	—	—	<0.01	0.8±0.1	—	0.07±0.03
BAL fluid[c]	—	—	4.3±0.6	2.7±0.4	—	15.9±2.5

[a]See Appendix A for abbreviations and acronyms; data (mean ± SE) extracted and summarized from Slade et al. (1985, 1993).
[b]Data expressed as mg/100 g wet tissue.
[c]Data expresses as nmol/mg protein.
[d]Data expressed as nmol/mg lipid phosphorus.

1979). In general, ventilation is reduced and changes in ventilatory patterns occur (Pavlin and Hornbein, 1986; Bellville et al., 1960; Hunter et al., 1968; Siafakas et al., 1983). In humans, anesthesia can decrease compliance and FRC, and it also can increase airway resistance (R_{aw}) (Rehder et al., 1974, 1975). In small laboratory mammals, an analogous decrease in FRC occurs, although apparently via a different physiological mechanism (Lamm et al., 1982). Additional anesthesia-related effects include a blockade of irritant reflexes (Weissberg et al., 1976) and alteration of ventilatory patterns in response to CO_2 (Martin-Body and Sinclair, 1985). Hence, although not invalidating experimental results, choice of anesthetic agent may affect the measured response and may confound cross-species comparison.

Restraint

Collection of small animal pulmonary function data without the use of anesthesia usually requires some type of physical immobilization. Restraint may range from minimally restrictive, allowing turning and some locomotion, to extremely confining, as occurs when animals are inserted into nose-only exposure tubes. Although restraint reduces movement

artifacts and permits attachment of delicate probes or sensors, immobilization can also produce undesirable physiological disturbances such as changes in body core temperature (T_{co}) (Nagasaka et al., 1979), hypermetabolism (Nagasaka et al., 1980), increased expiratory CO_2 (Jaeger and Gearhart, 1982), changes in ventilation and ventilatory pattern (Lai et al., 1978; Mauderly, 1986), and gastric response (Toraason et al., 1980). Such stress-related responses are poorly understood, and their influence on toxicologic responses may well pass unnoticed unless specifically examined.

Temperature

Although not widely appreciated, toxicant-induced changes in thermoregulatory function can modify the results of toxicological studies (Gordon et al., 1988; Gordon, 1991). Recent studies indicate that exposure to 0.37, 0.50, and 1.00 ppm O_3 also can decrease T_{co}, heart rate, and blood pressure over 2 or more hours in unrestrained, unanesthetized rodents maintained at normal room temperature (Uchiyama et al., 1986; Watkinson et al., 1995). On the other hand, when rats were restrained in a head-out body plethysmograph and exposed to the same concentration of O_3 (1 ppm) as in the Uchiyama et al. (1986) and Watkinson et al. (1995) studies, no change in blood pressure was observed (Tepper et al., 1990). The discordance between these findings may be the result of restraint stress, which has been shown to increase T_{co} (Nagasaka et al., 1979) and, in this circumstance, could have blunted the decrease in T_{co} associated with O_3 exposure.

Although O_3-induced changes in heart rate and T_{co} may be unique to rodents, this phenomenon has not been well studied in humans. It is possible that because of their larger thermal mass and different thermoregulatory mechanisms, humans do not exhibit similar changes in these parameters on exposure. For example, rectal temperature increased by the same amount in both air and 0.4 ppm O_3 groups of humans during a 2-h exposure at 35 °C (Bedi et al., 1982). The effects on T_{co} may have been confounded because the subjects performed moderate exercise during alternate 15-min periods during exposure. On the other hand, women exercising intermittently in moderate (24 °C) and hot (35 °C) ambient conditions showed no change in T_{co} related to O_3 exposure, but did show less of an increase in heart rate (2.7%) than did air-exposed (8.1%) subjects at 35 °C (Gibbons and Adams, 1984). It should be noted, however, that other studies have shown potentiation of human lung function responses associated with increased ambient temperature and O_3 exposure (Folinsbee et al., 1977; Gibbons and Adams, 1984). The full importance of temperature in relating rodent and human responsiveness to O_3 remains to be understood.

Exercise and Ventilation

Exercise has long been employed in human studies to enhance the effects of air pollutants, especially O_3 (Folinsbee and Raven, 1984). Exercise appears to exacerbate functional effects by increasing the inhaled dose (Hatch et al., 1994) and possibly by shifting the deposition of the pollutant to more sensitive pulmonary sites (Gerrity and Wiester, 1987). Although exercise can be used in laboratory animals to enhance deposition of O_3, no direct methods for measuring ventilation or breathing mechanics are available for small animals during exercise. Alternatively in an attempt to mimic the increase in ventilation produced by exercise in humans, studies employing restrained animals have used CO_2 as a ventilatory stimulant. Carbon dioxide (8 to 10%) maximally increases $\dot{V}_E$ three to five times in rodent species; CO_2 in excess of 10% will result in a reduction in ventilation (Wong and Alarie, 1982;

Tepper et al., 1988). This increase in $\dot{V}_E$ is equivalent to light (2 to 3 × resting $\dot{V}_E$) or moderate exercise (4 to 6 × $\dot{V}_E$) in humans (U.S. Environmental Protection Agency, 1986). In many O_3 studies in humans, both heavy (7 to 8 × $\dot{V}_E$) or very heavy (>9 × $\dot{V}_E$) exercise have been used. Similar increases in ventilation cannot be attained in small animals using the CO_2 challenge technique, thus posing a limitation in attempting to make direct comparisons between animal and human studies.

The application of this CO_2-challenge methodology in O_3-exposed rats (0.25 to 1.0 ppm for 2.25 h with 15 min alternating hyperventilation) clearly demonstrates enhanced pulmonary irritation, as reflected in breathing pattern changes during exposure (Tepper et al., 1988, 1990). The breathing pattern alterations typical of O_3 exposure appeared to be larger than would be predicted based solely on increased dose, suggesting that CO_2 challenge during O_3 exposure may have enhanced deposition at critical lung sites (Tepper et al., 1989). This augmented response was reflected clearly in the large increases in protein observed in the BAL fluid (Costa et al., 1988b). In postmortem studies, rats exercised during exposure have been found to have exacerbated lung pathology, thus appearing to confirm this hypothesis (Mautz et al., 1985) and suggesting that exercise may, in fact, enhance toxicity disproportionate to the apparent dose of toxicant.

8.3.3.2 Acute Exposure Data

Two corners of the parallelogram paradigm can be constructed readily from data gathered in empirical studies of acute O_3 exposure in humans and laboratory animals. These studies have the bulk of the data with the highest frequency of common endpoints that can be compared. Hence, the following discussion will focus on several categories of homologous lung function and BAL study data that have been obtained from humans and animals exposed similarly to O_3. The human studies were drawn from the large existing database on lung function and represent typical responses. The corresponding BAL data are more limited and are used to the extent possible. In contrast, the animal studies selected for comparison are highly selective and represent a rather small database involving similar exposure scenarios and homologous endpoints. This approach, of necessity, excludes the large majority of animal studies, not because they do not contain important toxicologic data on O_3, but rather, they are disparate in their exposure parameters or biologic endpoints that readily can be tied to those available in humans.

Tidal Breathing

In humans, O_3 produces pulmonary irritation, a response associated with cough and substernal soreness (Chapter 7). Although these symptoms are difficult to assess in animals, exposure to sufficient concentrations of O_3 produces reflex alterations in tidal breathing that can be measured objectively. Most notably, the response is an increase in f that is usually accompanied by a decrease in V_T (tachypnea), whereas $\dot{V}_E$ may not be altered. Although the magnitude of the tachypneic response is variable, depending on the species and exposure conditions, this endpoint is quite sensitive and consistent across many species (e.g., guinea pigs, cats, dogs, rats, monkeys, humans).

To examine the cross-species response to O_3, data were evaluated from human and animal studies that reporting immediate postexposure alterations in f. Most human studies employed an exercise regimen during O_3 exposure to increase dose. On the other hand, few animal studies have used exercise, relying rather on high exposure concentrations or

CO_2-induced hyperventilation. Three representative human studies were selected because they used a large range of concentrations and ventilation rates. Selected data from these three human studies are compared to the available animal data in Figure 8-7 and discussed further below.

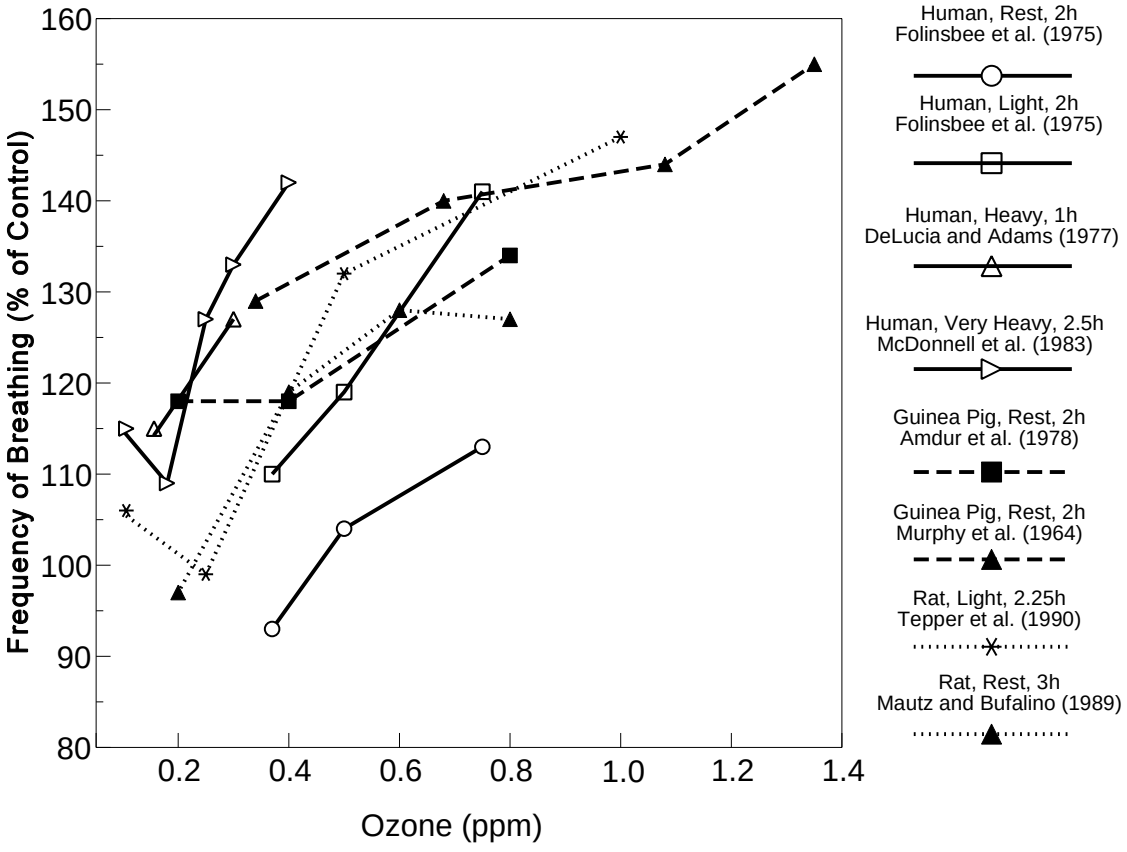


Figure 8-7. Comparison of changes in frequency of breathing after ozone exposure in humans and animals. Data are expressed as percent of the control response. Right-hand legend indicates species, exercise level, exposure duration, and the reference. Human data are plotted with solid lines and open symbols, whereas animal data are plotted with broken lines (differentiated by species) and closed symbols.

Folinsbee et al. (1975) examined the upper limits of the O_3 concentration-response curve in human subjects exposed either at rest or while performing light to moderate intermittent exercise (IE; 29 L/min; 5 × resting $\dot{V}_E$). Measurements of f were obtained during exercise following a 2-h exposure to 0.37, 0.50, or 0.75 ppm O_3. Concentration-dependent increases in f were observed in both the resting and exercising group, although the magnitude of the change in the exercising group was greater. The role of exercise in altering

the O_3-induced changes in f was examined further by DeLucia and Adams (1977), who exposed humans to 0.15 or 0.30 ppm O_3 for 1 h while the subjects exercised at one of four ventilation levels (1, 3, 4, or 6 × resting $\dot{V}_E$). The magnitude of the f response increased with concentration and exercise level, but was significant only in the highest exercise group at 0.15 and 0.30 ppm. Lastly, the lower limits of the concentration-response were explored by McDonnell et al. (1983), where subjects performing very heavy exercise (65 L/min; 10 × resting $\dot{V}_E$) were exposed to 0.12, 0.18, 0.24, 0.30, or 0.40 ppm O_3 for 2.5 h. Significant changes in f were observed at all exposure levels.

Although several animal studies have evaluated tidal breathing changes during and after O_3 exposure, only four studies have examined multiple concentrations such that comparisons to human data can be made. Unanesthetized, restrained guinea pigs were exposed for 2 h to 0.34, 0.68, 1.08, or 1.34 ppm O_3 via nose cones, while tidal breathing was measured using a constant-volume plethysmograph (Murphy et al., 1964). A similar experimental preparation was used by Amdur et al. (1978) to evaluate the respiratory response of guinea pigs to 0.2, 0.4, and 0.8 ppm O_3. In both of these experiments, a monotonic increase in f was observed; however, the animals studied by Murphy et al. (1964) were uniformly more sensitive to O_3 than those of Amdur et al. (1978). Mautz and Bufalino (1989) measured breathing patterns in awake, restrained rats exposed for 3-h to 0.2, 0.4, 0.6, and 0.8 ppm O_3. Concentration-related increases in f were observed up to 0.6 ppm, but the responses to 0.6 and 0.8 ppm were the same. In another study, awake rats were exposed to 0.12, 0.25, 0.50, and 1.00 ppm O_3 for 2.25 h in head-out pressure plethysmographs where CO_2-stimulated breathing was incorporated to augment ventilation (Tepper et al., 1990). With the added CO_2, rats and guinea pigs appeared to be similarly responsive to O_3. In general, as depicted in Figure 8-7, restrained guinea pigs and rats appeared to be as responsive as the lightly exercising humans, and clearly more responsive than the humans exposed at rest. Only with strenuous exercise does the response of humans appear to exceed that of rodents.

In addition to similar concentration-related effects in humans and animals, the time-related effects of O_3 exposure appear to be similar. To demonstrate this homology, Mauderly (1984) compared the time course of response to O_3 in humans and guinea pigs exposed under somewhat similar conditions. Humans were exposed to 0.75 ppm O_3 for 2 h while engaging in nonstrenuous IE at 15-min intervals (Folinsbee et al., 1975). In another study, respiratory parameters were measured at 30-min intervals during exposure and for 4 h postexposure (Bates and Hazucha, 1973). Similarly, unanesthetized, restrained guinea pigs were exposed to 0.68 ppm O_3 for 2 h as part of a concentration-response study (described above), with respiratory function assessed at 15-min intervals during exposure and for 3.5 h postexposure (Murphy et al., 1964). In both guinea pigs and humans, f increased and V_T decreased; both parameters then returned toward control values during the postexposure period. The percent change from control in f and V_T was nearly the same throughout the exposure and postexposure periods, indicating that a similar concentration of O_3 (0.7 ppm) produced similar temporal alterations in ventilation. Again, the guinea pigs would appear to be slightly more responsive than humans because the guinea pigs were exposed to a lower concentration (0.68 ppm) at rest, whereas the humans were exposed to 0.75 ppm with light IE.

Mechanics

Breathing mechanics have been examined in several animal and human-O_3 exposure studies, but there is little similarity between the databases for the concentrations or the specific

techniques used. Bates et al. (1972) examined breathing mechanics in resting and in lightly exercising (2 × resting $\dot{V}_E$) humans exposed for 2 h to 0.75 ppm O_3. Although no concentration-response data were obtained, increased total pulmonary resistance (R_L) and decreased dynamic compliance (Cdyn) were found for both resting (+22 and −12%) and exercising (+67 and −51%) subjects exposed to O_3. In a similar study, Hazucha et al. (1989) exposed men for 2 h to 0.5 ppm O_3 using moderate IE (40 L/min), and found a significant 12.5% increase in R_{aw}, although no concomitant change in Cdyn was detected. McDonnell et al. (1983), using a broad range of O_3 concentrations (0.12, 0.18, 0.24, 0.30, and 0.40 ppm; 2 h) and very heavy exercise (65 L/min), reported concentration-dependent increases in R_{aw}. In humans at rest or performing light exercise, however, a 2-h exposure at near-ambient O_3 concentrations would be expected to induce only modest increases in R_{aw} and no changes in Cdyn (Hazucha, 1987).

Although relatively high O_3 concentrations (≥1.0 ppm) produced effects on R_L and Cdyn in animals (Murphy et al., 1964), only three studies in animals have evaluated these parameters at lower, more relevant O_3 concentrations. Watanabe et al. (1973) studied anesthetized, paralyzed, and mechanically ventilated cats exposed via a steel tracheal tube to either 0.25, 0.50, or 1.00 ppm O_3 for between 2 and 6.5 h. Measurements of breathing mechanics were recorded every 30 min. With increasing O_3 concentration and exposure duration, R_L increased and, to a lesser extent, Cdyn decreased. Bronchoconstriction at 0.25 ppm O_3 was reversed following atropine (a parasympathetic receptor blocker), but only partially reversed at the two higher concentrations, suggesting the involvement of more than bronchoconstriction in the increase in R_L at these levels. Unfortunately, relating the concentrations used in this study to other animal or human studies is difficult because exposure through a tracheal tube would eliminate scrubbing of O_3 by the nose and oropharynx and likely would exaggerate the pulmonary O_3 dose (Gerrity et al., 1988).

Other studies have attempted to examine breathing mechanics in unanesthetized animals with natural nasal breathing and avoidance of potential anesthesia-related blunting of reflex responses. Murphy et al. (1964) exposed unanesthetized guinea pigs to several concentrations of O_3 for 2 h and measured ventilation, as previously discussed, and R_L. At concentrations less than 1 ppm, O_3 had no effect, but R_L increased 48 and 147% at 1.08 and 1.34 ppm, respectively. Using a similar test system, Amdur et al. (1978) observed no significant alteration of R_L in unanesthetized guinea pigs during a 2-h exposure to 0.2, 0.4, or 0.8 ppm O_3. However, Cdyn decreased significantly at 0.4 and 0.8 ppm O_3. In analogous studies in unanesthetized rats, Tepper et al. (1990) observed no significant changes in R_L or Cdyn after a 2.25 h exposure to 0.12, 0.25, 0.50, or 1.00 ppm O_3, in spite of intermittent 15-min periods of exercise-like hyperventilation induced by CO_2.

Although increased resistance is demonstrable in guinea pigs, cats, dogs, and humans, a comparison of percent change in resistance from control measurements after an acute (≤2 h) O_3 exposure (Figure 8-8) suggests that humans are more likely to bronchoconstrict due to O_3 exposure than rodents. Neither of the guinea pig studies (Murphy et al., 1964; Amdur et al., 1978) nor the rat study (Tepper et al., 1990) showed a significant increase in R_L at less than 1 ppm. However, closer examination of the human data reveals that the McDonnell et al. (1983) study employed very heavy exercise, and most of the studies included in the Hazucha (1987) model used moderate to heavy exercise. Thus, the inhaled dose likely would be greater than in spontaneously breathing animals. A more comparable study in humans that employed

only light exercise reported that 0.25, 0.37, and 0.50 ppm for 2 h resulted in minimal, nonsignificant 118, 124, and 104% increases in R_{aw} (Hackney

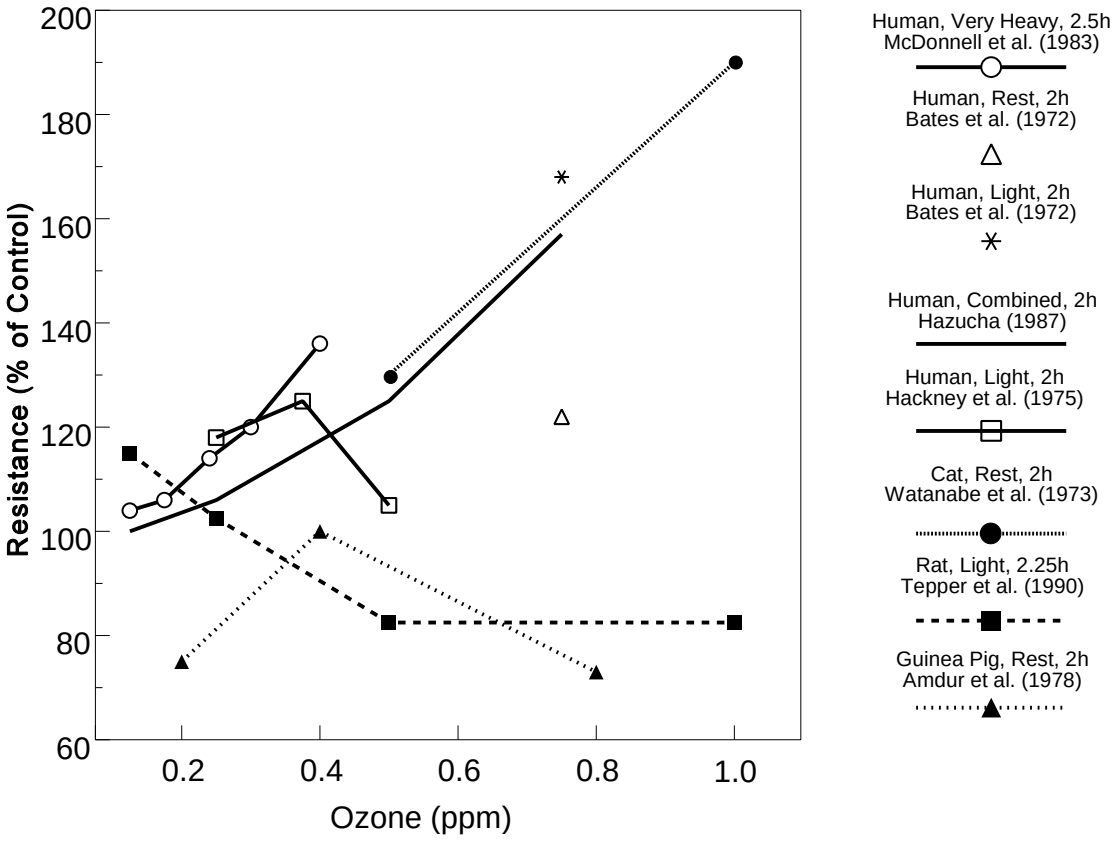


Figure 8-8. Comparison of changes in resistance after ozone exposure in humans and animals. Data are expressed as percent of the control response. Right-hand legend indicates species, exercise level, exposure duration, and the reference. Human data are plotted with solid lines and open symbols. The line labeled "Hazucha (1987)" is a model of predicted response. Animal data are plotted with dashed lines (differentiated by species) and closed symbols.

et al., 1975). Likewise, the Bates et al. (1972) data obtained in subjects at rest and with light exercise (0.75 ppm O_3) also argue against an unusually high O_3 responsiveness in humans relative to test animals for this endpoint when exercise-related dose is considered.

In general, similar findings have been observed using the measurement of Cdyn; however, the response decrements were more variable and of smaller magnitude. Given the distal deposition of O_3, as indicated by morphological studies (Chapter 6, Section 6.2.4), it is surprising that so little attention has been given to this parameter. Available data suggest that these changes in Cdyn are of little biological significance for ambient exposures.

Airway Responsiveness

The ability of O_3 to increase airway responsiveness to nonspecific bronchoconstricting stimuli in humans and other mammalian species has been known for at least a decade (Chapters 6 and 7, Sections 6.2.5 and 7.2.4, respectively). However, airway responsiveness is perhaps the least understood response to O_3, particularly in the context of species comparisons. Humans clearly exhibit increases in airway responsiveness at environmental O_3 exposure levels (Gong et al., 1986; McDonnell et al., 1987; Folinsbee et al., 1988; Horstman et al., 1990), whereas analogous responses in animals at O_3 concentrations below 1 ppm are controversial. Most studies of airway responsiveness in laboratory animals focus on the development of asthma-like models to elucidate generic mechanisms of airway responsiveness and utilize concentrations as high as 3 ppm for brief periods of time to injure the airways. Hence, anything more than a qualitative comparison between animal species and humans is tenuous and, thus, will not be discussed further in this section. Details of the methodologies of the laboratory animal and human bronchoreactivity studies can be obtained in the reviews of pulmonary function found in Chapters 6 and 7, Sections 6.2.5 and 7.2.3, respectively.

Elasticity and Diffusion

The integrity of the pulmonary air-blood barrier is essential for efficient exchange of oxygen and CO_2. This fragile epithelial interface with matrixed interstitial connective tissues and capillaries possesses inherent elastic properties and presents a finite resistance to oxygen diffusion to the blood. Although the elastic and diffusionary properties of the blood-air barrier are not linked implicitly to one another functionally, both properties can be quantified readily and compared between humans and laboratory animals (Costa, 1985). When combined, assessment of these functional properties is often sufficient to evaluate pathologic or toxic events in the distal reaches of the lung. For this reason and because of the fact that O_3 deposits in the deep lung, the effects of O_3 on these parameters will be discussed together.

Inhaled O_3 is known to penetrate to the depths of the lung and preferentially deposit in the smallest airways and its proximal acini (Section 8.2). Somewhat surprisingly, relatively few studies in humans have sought to characterize potential functional impairments at the air-blood interface. The reasons for this are likely twofold. First, in the early studies of the health effects of O_3 on humans, static compliance and diffusing capacity for carbon monoxide (DL_{CO}) were affected at only very high concentrations, well above what would be considered environmentally relevant. Second, from a practical perspective, these measurements proved to be considerably more tedious to perform than the forced expiratory measurement, which sensitively detects O_3-induced alterations (discussed below). Nevertheless, there are sufficient data on humans exposed acutely to O_3 to allow a reasonable comparison of these endpoints with their more abundant animal homologues.

The earliest studies leave little doubt that O_3 is edemagenic at high concentrations in virtually all mammalian species. In the past, occupational exposures of 2 to 3 ppm O_3 were not uncommon, and a 9-ppm peak exposure has been reported (Kleinfeld et al., 1957; Challen et al., 1958). The resultant worker symptoms and signs, including chest radiograms, were consistent with the manifestations of edema reported in experimental animals (i.e., increased lung weight and stainable edema in the airspaces) (Stokinger, 1965). Lung function, however, typically was not measured in these work-related exposures. In a later study of arc welders exposed to 0.2 to 0.3 ppm O_3, little, if any, convincing evidence of functional impairment, in

terms of altered lung volumes, maximal expiratory flow rates, and DL_{CO} was obtained (Young et al., 1963). To further explore the possible effects of O_3, Young and coworkers (1964) subjected 11 human volunteers at rest to controlled atmospheres of 0.6 to 0.8 ppm O_3 via mouthpiece for 2 h. Small reductions in vital capacity (VC), forced expiratory volume in 0.75 s, dynamic and static lung compliance, and intrapulmonary gas distribution were observed, but only the 25% fall in DL_{CO} proved to be statistically significant. Similar, but considerably more variable effects on lung function were reported by Hallett (1965) in 10 subjects exposed to 1 to 4 ppm O_3 for 30 min. Nonetheless, of 10 exposed subjects, seven showed at least a 20% drop in DL_{CO}. Like Young and coworkers, Hallett (1965) interpreted these changes to indicate lung edema, in agreement with the hypothesis that the deep lung irritant O_3 was having its effect at the alveolar level. Interestingly, additional work from the same laboratory of the Young study (Bates et al., 1972) found that resting subjects receiving nasal exposure to 0.75 ppm O_3 for 2 h resulted in a nonsignificant 3% reduction in DL_{CO}. However, in a limited test group, the co-imposition of light exercise, which doubled ventilation, enhanced this response (□12%). It appears that the nasal (Bates et al., 1972) versus mouthpiece (Young et al., 1964) routes of exposure were instrumental in the differential response, because it is likely that the mouthpiece diminished what scrubbing occurs when exposure is via the unencumbered mouth in human test subjects (as reported by Gerrity et al., 1988). Since these early studies, there have been no additional controlled human acute studies that have examined alterations in DL_{CO} at O_3 concentrations below 0.6 ppm.

Analogous animal studies of acute O_3 exposure indicate that the general pattern of functional impairment is similar to that reported in human studies. Anesthetized and ventilated cats showed a general decline in VC, static lung compliance, or DL_{CO} with exposures up to 6.5 h of 0.26 to 1.00 ppm O_3 (Watanabe et al., 1973). The responses of the 20 animals were variable, and these declines, which did not achieve overall statistical significance, were thought to be largely secondary to the substantial (36 to 200%) increases in R_{aw}. In a more complex study design, rats were exposed for 2 or 7 h to 0.5 or 0.8 ppm O_3 with intermittent 8% CO_2 to hyperventilate (□2 to 3 × resting $\dot{V}_E$) the animals as an exercise analogue to human exposures (Costa et al., 1988a). The DL_{CO} values were reduced by about 10% at both 0.5 ppm time-points and by about 12% with a 2-h exposure to 0.8 ppm. Exposure to 0.8 ppm for 7 h, however, greatly exacerbated the alveolar effect with a resultant 40% reduction in DL_{CO}. Static compliance, unaffected by the other exposure conditions, was affected only at this latter exposure duration. These O_3-induced effects, particularly the reductions in DL_{CO}, appeared to correlate with the degree of lung edema in affected animals, as had been surmised for the acutely exposed humans. With the multitude of more recent studies of O_3 at ambient levels, alterations in static lung compliance or DL_{CO} rarely are reported in either humans or animals.

Forced Expiration

Reductions in FVC and FEV_1 have become the hallmarks of acute lung dysfunction in humans after O_3 exposure (Chapter 7). These measures are sensitive to O_3 levels as low as 0.12 ppm for as little as 2 h when heavy IE is included during the exposure (McDonnell et al., 1983) and show cumulative dysfunction resulting from 6.6 h of lower levels of this oxidant (0.08 and 0.10 ppm) when nearly continuous, moderate exercise is employed (Horstman et al., 1990). Reductions in FEV_1 and FVC induced by O_3 are believed to be partly the result of pain-mediated interruption of maximal inspiration (Hazucha et al., 1989). Exactly what level

of tissue injury or inflammation correlates with these functional deficits is unclear and is an active area of research (Chapter 7, Section 7.2.4).

Most studies of O_3 in experimental animals make little effort to mimic human study designs, thereby impeding the extrapolation of experimental animal study results to humans. Recently, however, rat studies involving periods of intermittent CO_2-induced hyperventilation to enhance dosimetry have attempted to capitalize on the qualitative similarity of the rat and human maximum expiratory flow volume (MEFV) curves as a potentially sensitive endpoint of toxicity (Costa et al., 1988a; Tepper et al., 1989). In the rat, FVC does indeed decrease with O_3 exposure, although the magnitude of response is apparently less than that observed in humans. As in the human, the reduced FVC in the animal appears not to be the result of a change in residual volume. Total lung capacity may be reduced slightly, but lung compliance does not change. However, it is premature to assume a common mechanism for the FVC reductions in the rat and human. Unlike the human, pain on inspiration in the animal model is likely not an issue because the animal is anesthetized during the procedure and is brought to TLC by a defined airway pressure (30 cm H_2O). Because general anesthesia is known to diminish sensory afferent stimuli, an analogous O_3-induced fall in rat FVC should expectedly have been blunted, if not totally eliminated. To what extent anesthesia mitigates the rat response or that there are inherent species differences in dosimetry or sensitivity is not clear from these studies. Nevertheless, comparison of model-predicted FVC changes in humans (Hazucha, 1987) with analogous rat data (Costa et al., 1988a) would suggest that this response in the anesthetized rat is about half that of the human (Figure 8-9).

Studies of Inflammation and Antioxidant Content of Bronchoalveolar Lavage Fluid

Both humans and animals exhibit a PMN inflammatory response with associated changes in lung permeability after acute exposure to O_3. Recent studies indicate that humans exposed to O_3 concentrations as low as 0.08 ppm for 6.6 h with moderate exercise (40 L/min) exhibit a fourfold increase in the percentage PMNs when BAL is obtained 18 h postexposure (Devlin et al., 1991). To date, animal studies at comparable exposure levels are rare, (Hotchkiss et al., 1989), and exercise enhancement of exposure dose has yet to be incorporated. As noted above, the issue of dosimetry is critical if extrapolation at such levels is to be attempted. Nevertheless, in the one acute rat study at 0.12 ppm O_3 for 6 h, an increase in nasal-lavage-derived PMNs was noted 18 h postexposure, with no similar change in PMN number in the BAL (Hotchkiss et al., 1989). In contrast, in the same study when higher concentrations of O_3 (0.8 and 1.5 ppm) were used, BAL PMNs were elevated, but no changes were observed in the nose washings. Such a "competitive" nasal-pulmonary response has yet to be studied directly in humans. Nevertheless, the data support the general hypothesis that there is comparability between the inflammatory responsiveness of rats and humans. More direct comparison of laboratory animal inflammatory responses with those of humans can be drawn from studies at higher concentrations when the nasal/lung competitive response in the rat is skewed to the lung, and, like in the lung-function comparison, analogous exposure conditions can be more directly compared. These studies are tabulated in Table 8-9 and discussed in more detail below.

Four representative human studies of lung inflammatory responses after acute O_3 exposure can be compared with existing acute animal data from studies of analogous design. Seltzer et al. (1986) exposed moderately exercising (83 to 100 W) subjects to 0.4 or 0.6 ppm O_3 for 2 h, with BAL obtained 3 h postexposure. The BAL fluid from the

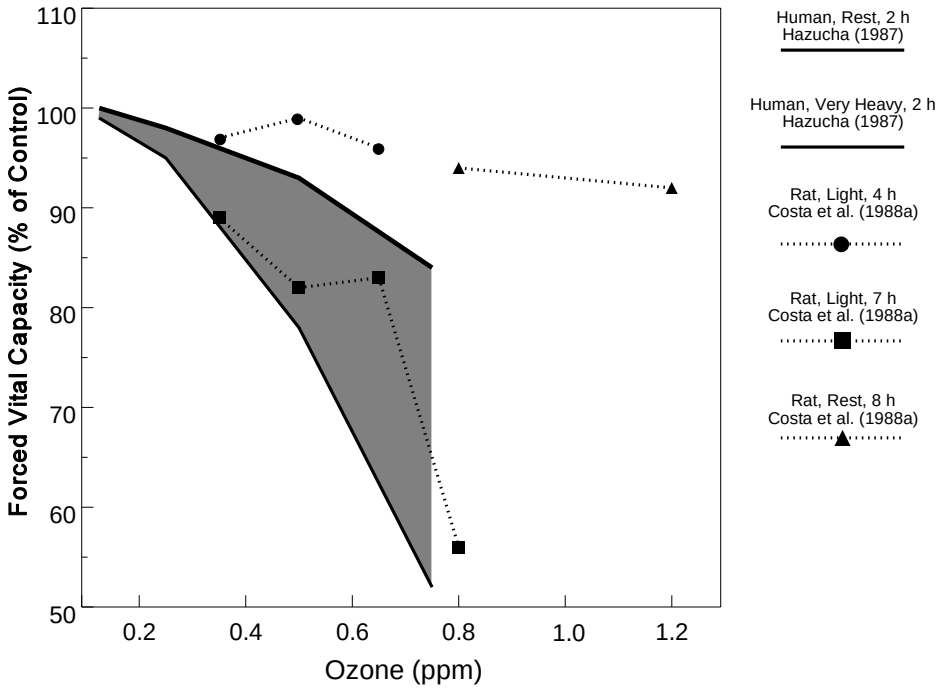


Figure 8-9. Comparison of changes in forced vital capacity after ozone exposure in humans and animals. Data are expressed as percent of the control response. Right-hand legend indicates species, exercise level, exposure duration, and the reference. Human data are plotted with solid lines from the equation of Hazucha (1987), with the shaded area representing the predicted range of decrements expected between light exercise (top line) and very heavy exercise (bottom line). Rat data are plotted with dashed lines and closed symbols.

combined 0.4- and 0.6-ppm O_3 groups contained an average 7.8-fold more PMNs than did BAL fluid after filtered air. Protein levels in the BAL fluid were not assayed in this study. In separate studies, humans were exposed to 0.4 ppm O_3 for 2 h with heavy exercise (60 L/min), with BAL samples collected at 1 and 18 h postexposure (Koren et al., 1989b, 1991). These two studies showed that the inflammatory response is quickly initiated postexposure. The PMN and protein content were elevated at both times (1 h: PMN 18.2×; protein 1.2×), with the 18-h timepoint (PMN 8×; protein 2.2×) being higher for protein, but somewhat less for PMNs. Schelegle et al. (1991) followed the time course of the inflammatory response (1, 6, and 24 h postexposure) after a 1-h exposure of 0.3 ppm O_3 with exercise (60 L/min). The PMN content of the combined airway and alveolar BAL fluid was elevated at 6 h (3×) and 24 h (2.5×) only, with the 6-h point representing the peak. Protein content of the BAL fluid did not change significantly at any point.

The many studies of O_3-induced inflammation in laboratory animals are reviewed in Chapter 6, Section 6.2.2. For the purpose of species comparison, only selected studies

Table 8-9. Polymorphonuclear Leukocyte and Protein Permeability Response to Ozone by Species[a]

Exposure Parameters	Exposure Conditions	Species (Strain)	Postexposure BAL Time	PMN[b] Increase	Protein[b] Increase	Reference
0.4-0.6 2 h	Exercise (15 min/alt; 83-100 W)	Human	3 h	7.8	Not done	Seltzer et al. (1986)
0.4 2 h	Exercise (15 min/alt; 60 L/min)	Human	18 h	8.0	2.2	Koren et al. (1989a,b)
0.4 2 h	Exercise (15 min/alt; 60 L/min)	Human	1 h	18.2	1.2	Koren et al. (1991)
0.3 1 h	Exercise (15 min/alt; 60 L/min)	Human	1, 6, 24 h	3.0 at 6 h 2.5 at 24 h	No change	Schelegle et al. (1991)
0.96 8 h	Daytime; rest	Rhesus monkey	1, 12, 24, 72, 168 h	27 at 1 h 19 at 12 h 24 at 24 h 6 at 72 h 3 at 168 h	3 at 1 h 3 at 12 h 8 at 24 h 3 at 72 h 1 at 168 h	Hyde et al. (1992)
0.2 0.5 1.0 2.0 4 h	Daytime; rest	(a) Mouse (Swiss Albino) (b) Guinea pig (Hartley) (c) Rat (S-D) (d) Hamster (Golden Syrian) (e) Rabbit (NZW) M	16 h	Not done	(a) 1.8 at 1.0 ppm 3.2 at 2.0 ppm (b) 1.4 at 0.2 ppm 2.0 at 0.5 ppm 4.1 at 1.0 ppm 4.5 at 2.0 ppm (c) 2.1 at 1.0 ppm 3.6 at 2.0 ppm (d) 1.5 at 1.0 ppm 2.6 at 2.0 ppm (e) 2.7 at 2.0 ppm	Hatch et al. (1986)
0.5 1.0 4 h	Daytime; rest; Vitamin C (AH_2+/□)	Guinea pig (Hartley) M	16 h	Not done	For 0.5 ppm; 1.1 for AH_2^+, 2.1 for AH_2□. For 1.0 ppm; 2.4 for AH_2^+, 2.7 for AH_2□	Slade et al. (1989)
0.5 0.8 2 or 7 h	Daytime; (15 min/alt for 2 h; 45 min/alt for 7 h); 3-5× V_E	Rat (F344) M	1 h	Not done	For 0.5 ppm; 1.2 at 6 h, 2.1 at 7 h. For 0.8 ppm; 1.5 at 2 h, 3.3 at 7 h	Costa et al. (1988a)

[a]See Appendix A for abbreviations and acronyms.
[b]Ozone response/air response.

(Table 8-9) will be considered. The spectrum of exposure conditions used in the various animal studies makes difficult the direct comparison among laboratory test species. However, one study by Hatch and coworkers (1986) specifically addressed this question by exposing mice, guinea pigs, rats, rabbits, and hamsters under identical conditions (0.2, 0.5, 1.0, and 2.0 ppm O_3 for 4 h), followed at 18 h postexposure with BAL and assay for protein. Guinea pigs were most responsive, responding at 0.2 ppm, whereas mice, hamsters, and rats began responding at 1.0 ppm, and rabbits responded only to 2.0 ppm. Only one study involving BAL assessment of PMNs and protein in exposed monkeys has been published (Hyde et al., 1992). At 0.96 ppm O_3 (8 h), the monkeys had a significant inflammatory response, but it is difficult to assess monkey responsiveness relative to the human for this endpoint. Assuming a linear concentration times duration relationship, the monkey data appear similar to the guinea pig response. However, none of these species showed BAL protein increases approximating those reported in human studies.

In recent studies (0.4 ppm O_3 for 2 h, with BAL 16 to 18 h postexposure) (Slade et al., 1989; Crissman et al., 1993), guinea pigs made vitamin C-deficient exhibited enhanced responsiveness to O_3, this result is comparable to that of the exercised humans of Koren et al. (1989b) (Figure 8-10). Similarly, when rats were exposed to 0.5 ppm O_3 for 2 h with intermittent CO_2-induced hyperventilation (Tepper et al., 1993) to mimic mild/moderate exercise (three- to fivefold $\dot{V}_E$), the BAL protein, as well as PMN responses at 18 h postexposure compared favorably with those data of Koren and coworkers (1989a,b) (Figure 8-11).

Within a given laboratory animal species, responses among strains also can differ appreciably, as demonstrated in rats by Pino et al. (1991) and Costa et al. (1993). These studies indicated that Wistar rats exhibit greater inflammatory responses (protein and PMN) to O_3 than S-D and F344 rats after an 8-h exposure to 0.5, 1.0, and 1.5 ppm with BAL sampled at 2 or 24 h later. Similarly, mouse strains (C3H/HeJ and B6C57/6; Kleeberger et al., 1990) and S-D rat substrains (Costa et al., 1985) have been shown to possess specific genetic susceptibility to high levels of O_3 (2 ppm). In the case of the mice, the responsive strain is seven times (at the 6-h postexposure peak) as susceptible as the databases in animals and, particularly, in humans with regard to these antioxidants are quite limited. Supplementation and deprivation studies with vitamins C and E have shown that these antioxidants have some role in protecting against the effects of O_3 in animals (Elsayed et al., 1988; Slade et al., 1989; Crissman et al., 1993) and in humans (Chatham et al., 1987). Of the animal models, ascorbate-deprived guinea pigs appear to have BAL ascorbate levels most like humans, with a protein permeability response (without exercise in the animal) very similar to the human exposed to the same concentration (0.4 ppm O_3 for 2 h) with exercise. However, Crissman et al. (1993) also resistant strain for the PMN response to 2 ppm O_3; the protein response is twice (at 24 h postexposure) that of the resistant strain. In the S-D substrain, protein extravasation into the alveolar lumen immediately postexposure is 40% higher in the responsive strain than the resistant (no other time points were examined).

Humans have an order of magnitude less ascorbate in BAL fluid as compared to the rat, but they have nearly twice the glutathione, four times the uric acid, and 80 times the vitamin E, as normalized to lipid P-surfactant (Slade et al., 1993) (Table 8-8). However, on a BAL-derived cell/protein basis, the ratios clearly favor the rat for all of these antioxidants, with the exception of uric acid, which is generally not high in rats because of species differences in protein and prime nucleotide catabolism (urea being the major nitrogenous

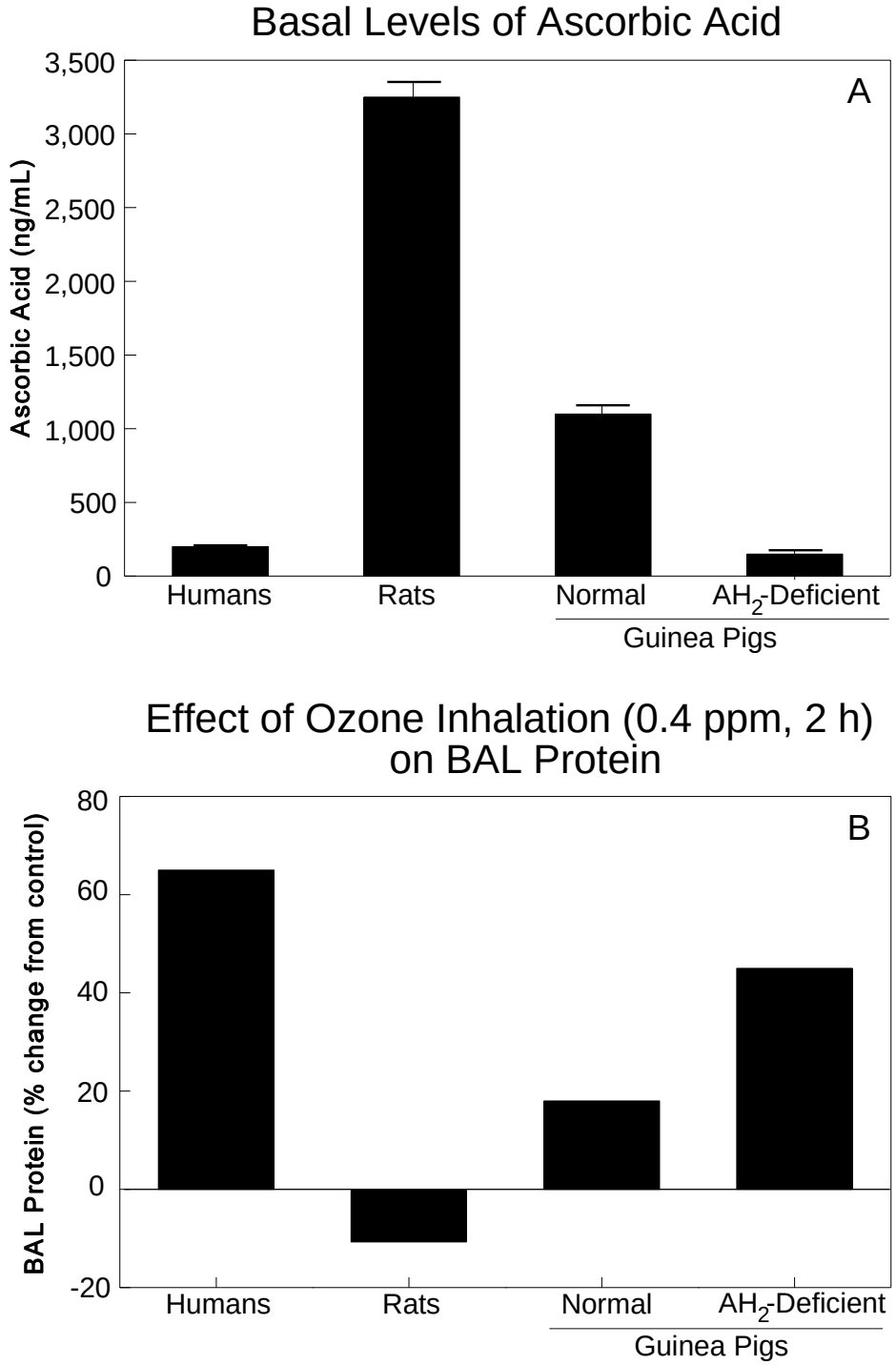


Figure 8-10. Composite of data from Slade et al. (1989), Koren et al. (1989b), and Crissman et al. (1993) comparing basal bronchoalveolar lavage (BAL) ascorbate levels (A) to ozone-induced changes in BAL protein (B). Ozone-exposures (0.4 ppm; 2 h; 16 to 18 h BAL) of humans (exposed with exercise), rats (exposed resting), and guinea pigs (exposed resting) with (ascorbic acid [AH_2]-deficient) and without (normal) AH_2 deficiency.

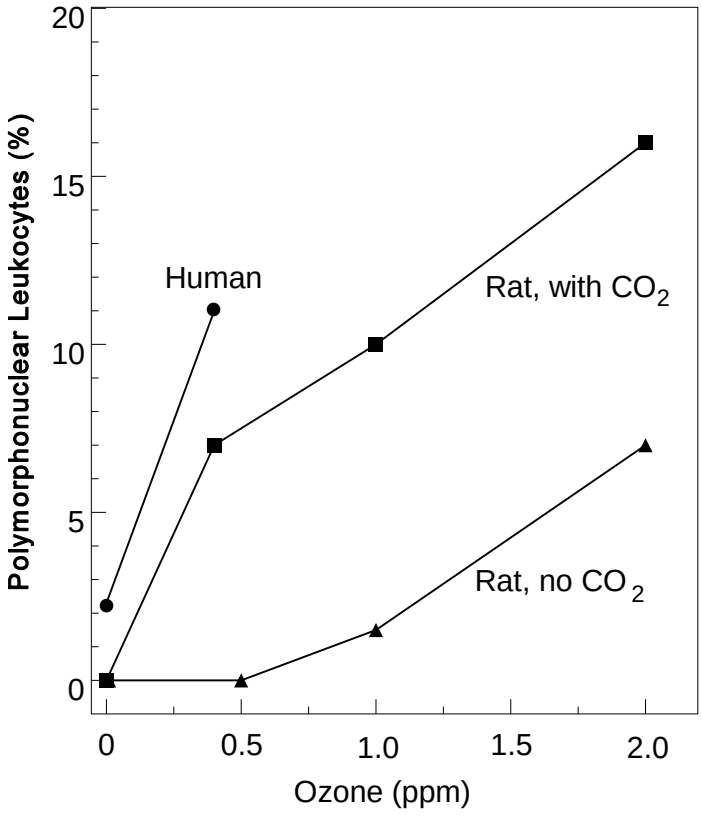


Figure 8-11. Composite of data comparing polymorphonuclear leukocytes obtained by bronchoalveolar lavage 16 to 18 h after ozone (O_3) exposure (0.4 ppm; 2 h) of humans (with exercise) (Koren et al., 1989b) to those of rats exposed to O_3 (0.5 to 2.0 ppm) at rest or hyperventilated with carbon dioxide (CO_2) (Tepper et al., 1993).

by-product for rats). The guinea pig most closely resembles the human for ascorbate. Because these antioxidants are thought to function in the defense against oxidant challenge, it would appear critical to appreciate their presence and function when attempting to interpret data for extrapolation. Unfortunately, the reported that reduced ascorbate levels in BAL fluid (18 h postexposure) increase in the human, whereas those levels decrease in the rat. Whether this relates to the distinctly different basal levels of this vitamin and is associated with the disparate protein responsiveness (ignoring exercise) is unclear because deficiency in animals (guinea pigs) appears more critical to the responsiveness at low (ambient-like) concentrations than at higher concentrations (1.0 ppm). Although it would appear that vitamin C is involved in the interplay between O_3 and the exposed subject (human or animal), there is not full coherence of the data. For example, Hatch et al. (1986) showed that the rabbit was the least responsive to O_3 in terms of BAL protein, but this species has among the lowest tissue levels of vitamin C (Slade et al., 1985). However, rabbits apparently have a low propensity to form lipid

peroxides (Arakawa et al., 1986), an expected product of lung lipid-O_3 interaction (Pryor, 1992). Thus, to interpret interspecies sensitivity only in terms of basal antioxidant levels, however tempting this may be, would be overly simplistic and premature at this time.

8.3.3.3 Prolonged Exposure Studies

The previous sections of this text have utilized lung function responses to acute O_3 exposure in an attempt to elucidate the relative sensitivity among laboratory test species and humans and, thereby, to complete two corners of the parallelogram paradigm (Figure 8-6). Because acute responses represent only part of the extrapolation paradigm, temporal-based exposure responsiveness also will be considered, despite the relative paucity of comparative data between laboratory animals and humans for prolonged exposures. Again, in an effort to best extract species-specific response differences, the criterium for selection of studies was similarity in exposure scenario. The discussion will focus on relative adaptability of acute functional changes and associated BAL-derived findings after repeated exposures and the coherence of the findings from prolonged human and animal exposure studies and epidemiological results.

Lung Function Studies

Reversal ("attenuation") of pulmonary function decrements using a scenario of repeated exposure to O_3 has been reported for both humans and laboratory animals. At least nine studies between 1977 and 1984 have documented that, for repeated exposures between 0.2 and 0.5 ppm O_3 (2 h/day, up to 5 days), spirometric changes were most severe on the first or second day of exposure, waned over the next 3 days of exposure, and, by the fifth day, had returned to control preexposure levels (Chapter 7, Section 7.2.1.4). In the only animal study using a similar exposure protocol and analogous experimental design, Tepper et al. (1989) showed that rats initially displayed a tachypneic response to O_3 that attenuated after 5 consecutive days of exposure, a pattern quite similar to that of humans. Exposures were for 2.25 h and included challenge with CO_2 during alternate 15-min periods to augment ventilation (2 to 3 $\times$ resting $\dot{V}_E$, which is equivalent to light exercise in humans). As in the human studies, the functional changes were largest on Day 1 or 2, depending on the parameter and the O_3 concentration (0.35, 0.50 and 1.00 ppm). Attenuation of the changes in shape constant of the flow-volume curve of the rats also was observed over this period. Thus, under analogous conditions of exposure, both the humans and the rats exhibited similar initial functional responses to O_3 with full and kinetically similar reversal of effects.

More difficult is the direct comparison between human and animal lung function responses to prolonged (several-week) O_3 exposure, largely because of the limited availability of controlled human study data. In the only study of its kind, Bennett (1962) exposed 12 human subjects at rest to 0.2 or 0.5 ppm O_3 for 3 h/day, 6 days/week for 12 consecutive weeks. Although no effects were discernable early in the exposure, there appeared to be small, but significant O_3-induced reductions in FEV_1 (and small, nonsignificant reductions in FVC), particularly in the last weeks of the study. This reduction in FEV_1 more likely would reflect obstructive changes within the lung at these points late in time rather than the pain-mediated reductions that are seen with acute O_3 exposure, which attenuate after a few days of exposure. The lack of concentration-related decrements in FEV_1 and FVC is somewhat unsettling, but, regardless, after 9 postexposure weeks in clean air, all measured effects had reversed.

Unfortunately, there are no directly parallel animal studies to compare to this limited database. But, if the two-fold sensitivity difference between the rat and human FVC response (see Figure 8-12) is assumed, a number of animal studies may be considered comparable for the purposes of this discussion. On the one hand, rats exposed to 0.2 or 0.8 ppm O_3 for 6 h/day, 5 days/week for 12 weeks were reported to exhibit some degree of small airway obstruction based on the MEFV curves, but little, if any, reduction in FVC or DL_{CO} was observed (Costa et al., 1983). Others have reported analogous marginal increases in rat TLC or its component volumes (Bartlett et al., 1974; Costa et al., 1983; Raub et al., 1983) or in regional R_{aw} (Yokoyama et al., 1984) after intermittent or continuous exposures to 0.25 ppm O_3 for 4 to 12 weeks, which would not be unexpected with distal airway or lung damage. Actual pathology in the distal lung tends to be focal and difficult to correlate precisely with the marginal functional impairment.

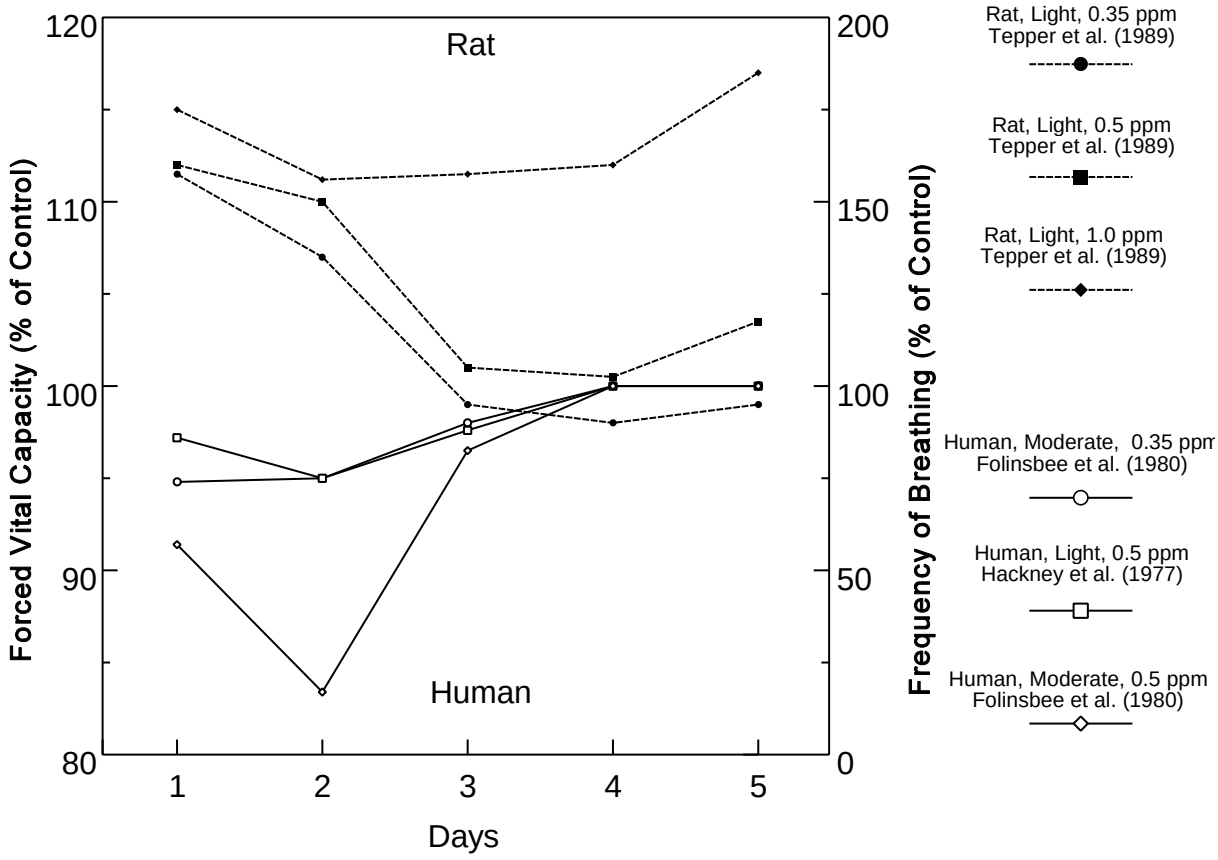


Figure 8-12. Comparison of changes in forced vital capacity in humans (left ordinate) and frequency of breathing in rats (right ordinate) with up to 5 consecutive days of ozone (O_3) exposure. Data are expressed as percent of the control response. Right hand legend indicates species, exercise level, O_3 concentration, and the reference. Human data are plotted with solid lines and open symbols, whereas rat data are plotted with broken lines and closed symbols.

The limited functional data available in monkeys generally agree with the pattern of distal lung pathophysiology reported in rats. When exposed to 0.5 ppm O_3 for 90 days (8 h/day), monkeys exhibited a slight increase in lung distensibility (Eustis et al., 1981). Likewise, monkeys exposed to 0.25 ppm (8 h/day for 18 mo) exhibited increased chest wall (but not lung) compliance and lung volumes, which was most marked in the monkeys exposed to O_3 only in alternate months during that 18-mo period (Tyler et al., 1988). Recall, however, that little or no change in lung elasticity has been associated with controlled O_3 exposures in humans, whether the exposures are acute or repeated. On the other hand, higher concentrations (0.64 ppm for 1 year) resulted in the alteration of distal airway mechanics in exposed monkeys, as gleaned from local resistances measured using oscillatory methods; again, this is in general agreement with the presence of distal lung injury or disease. Morphometric analyses of the end-airways and distal lung regions of O_3 exposed monkeys consistently show altered cell profiles and interstitial restructuring, even when functional changes are marginal, which, like in the rat, likely reflects the large functional reserve of the integrated lung. Thus, although these collective data from subchronic animal studies suggest a reasonably homologous distal lung response to O_3, many of these linkages in functional outcomes remain uncertain in terms of what to anticipate in the human response.

Clearly, the question of potential lung impairment resulting from a near-lifetime exposure to O_3 ranks among the most pressing concerns about this toxicant. The animal data, although demonstrating that chronic O_3 exposure can induce changes in the structure and function of the lung, have yet to provide evidence of potential disease or disability in humans exposed to O_3 over prolonged periods of their lives. The existing epidemiologic studies (Chapter 7, Section 7.4.2), too, merely provide suggestive evidence that persistent or progressive deterioration in lung function may be associated with long-term oxidant pollutant exposure (Detels et al., 1981, 1987). Detels and coworkers (1991) reported decrements in FEV_1 and nitrogen washout across all age groups in areas where oxidant pollution is high. Similarly, analysis of the pulmonary function data from the National Health and Nutrition Examination Survey (NHANES) II showed loss of lung function when annual averages ofambient O_3 exceeded 0.04 ppm (Schwartz, 1989). This pattern of impairment is consistent qualitatively with the chronic animal studies (Costa et al., 1994).

Studies of Inflammation and Antioxidant Content of the Bronchoalveolar Lavage Fluid

The virtual absence of human BAL study data after repeated or prolonged exposures to O_3 hinders the comparison of nonacute human and animal inflammatory responses. However, the recent study of Devlin et al. (1995) suggests that the PMN and protein responses to repeated daily exposures to 0.4 ppm O_3 (2 h with IE for 5 consecutive days) attenuate, much as do the functional responses. Hence, for most of the BAL parameters (with the exception of lactate dehydrogenase activity [a marker of cell injury]), there is indeed an apparent reversal of acute inflammation when exposures are continued over the 5-day exposure period. Rat studies largely appear to show similar attenuation to O_3, but this response seems to be influenced by exposure patterns or conditions (Bassett et al., 1988; Tepper et al., 1989; Van Bree et al., 1989). The study with the most similar design to the human protocol (Tepper et al., 1989) showed some reduction in BAL protein with repeated exposure involving intermittent hyperventilation with CO_2, but over the 5-day period, the protein levels remained significantly elevated; cells were not evaluated in this study. Interestingly, vitamin C and glutathione levels

in the BAL fluid increased over the 5-day course of exposure, a response consistent with an unregulated antioxidant role in the adaptative mechanism. Van Bree and coworkers (1992), on the other hand, reported that 5 consecutive days (12 h/day) of 0.4 ppm O_3 resulted in complete reversal of both BAL albumin and PMN measures to the control values. It should be noted, however, that other biomarkers and mediators within the BAL were not fully recovered, which might suggest a slower reversal time frame or continued O_3-induced pathogenesis, a conclusion of the Tepper et al. (1989) study.

In guinea pigs made deficient in vitamin C and exposed to O_3 (0.2, 0.4, or 0.8 ppm) continuously for 7 days, attenuation of the functional and inflammatory endpoints appeared nearly complete in spite of the deficiency (Kodavanti et al., 1995). Other antioxidants, not altered basally, were unregulated more by the O_3 challenge; the small residual reservoirs of ascorbate, which persisted in the nearly 98% deficiency state of the animals, were apparently mobilized to the site of injury, allowing repair to proceed. Likewise, chronically exposed rats have elevated BAL ascorbate indicative of the oxidant burden and the ongoing repair (Grose et al., 1988). Prolonged exposures up to 18 mo appear to sustain a low-grade interseptal inflammation and evidence of lung matrix remodeling in both rats and monkeys, suggesting that humans would behave similarly. However, such data are not presently available from humans.

8.4 Quantitative Extrapolation of Acute Ozone Effects

8.4.1 Introduction

Advances in dosimetry since the previous O_3 criteria document (U.S. Environmental Protection Agency, 1986) fall into five major areas: (1) greater sophistication of model applications (e.g., Overton et al., 1989; Mercer et al., 1991), (2) the appearance of experimental uptake data that can be compared to model predictions (Wiester et al., 1987, 1988, 1996; Hatch et al., 1989; Gerrity et al., 1988, 1994, 1995), (3) experiments specifically designed to estimate model parameters (Hu et al., 1992b), (4) a better understanding of the role of O_3 in the liquid linings and tissues of the RT (Pryor, 1992), and (5) a better understanding of anatomical aspects (Mercer et al., 1991). The role of these advances in interspecies dosimetric extrapolation follows.

With the information available for rats, reasonably reliable predictions of the flux of O_3 to the air-liquid lining interface of toxicologically important regions, such as the CAR, is possible. There are two main investigations that make this feasible: (1) Hatch et al. (1989), who estimated the percent uptake and the fraction of the retained O_3 that is in the URT, trachea, and lung of rats, and (2) Pinkerton et al. (1992) (with elaboration by Miller et al., 1993) who illustrated the basic correctness of modeling assumptions for ventilatory units. (The judgment of basic correctness is based on the assumption that the dose causing the response is proportional to the flux of O_3 to the air-liquid lining interface.) Using this information, regional mass transfer coefficients could be estimated, which would allow the prediction of local respiratory tract O_3 doses in rats exposed under general conditions.

The results of the investigation of Hu et al. (1992b) can be used to estimate URT and TB model parameters, but may not be sensitive enough to determine pulmonary region parameters. If uptake is not confined to the URT and TBs, then their mass transfer coefficients alone would not be sufficient to account for total RT uptake (e.g., as measured by Wiester et al. [1996] or Gerrity et al. [1988]), and the difference in predicted (without pulmonary

region uptake) and experimental uptakes could be used to estimate the pulmonary region mass transfer coefficient. Unfortunately, no dosimetry data for the human pulmonary region are available.

Despite limitations, the O_3 dosimetry data that have been obtained over the past several years, coupled with the advances in modeling, suggest that there has been continual convergence between the model predictions and experimental observations. Given the many areas of consistency between models and experiments, it is valuable to begin to employ these models to provide the dosimetric basis for animal-to-human extrapolation. One of the greatest sources of uncertainty in such an application of dosimetry models is the lack of full understanding of the appropriate target site for O_3 toxicity (e.g., upper or lower airways or pulmonary region) that initiates a particular response, especially the functional changes in the lung. However, reasonable assumptions can be made to narrow the target site.

The first application of dosimetry models given in Section 8.4.2 is an examination of delivered dose versus response within a given species. This is followed in Section 8.4.3 by some interspecies comparisons of delivered dose versus response.

8.4.2 Intraspecies Delivered Dose Response

Assuming that changes in FEV_1 in humans exposed to O_3 mainly are the result of O_3 pulmonary tissue dose, Miller et al. (1988) constructed a dose-response curve. They plotted decrements in FEV_1 versus predicted cumulative pulmonary region O_3 tissue dose scaled to body mass (Figure 8-13). The concentration-response data are from McDonnell et al. (1983), in which 135 healthy subjects were exposed to 0, 0.12, 0.18, 0.24, 0.30, and 0.40 ppm O_3. Exposure was for 2.5 h with heavy IE. Miller et al. (1988) used the average weight and height of the subjects to estimate the FRC that was used in the model to simulate O_3 dose. The exercise breathing parameter data were used along with an estimate of resting breathing parameters. Figure 8-13 is similar in shape to the concentration-response curve of McDonnell et al. (1983). Differences between these two curves, however, are accounted for by the translation between exposure concentration and O_3 dose.

As another example, Pinkerton et al. (1992) examined the relation between actual tissue response of rats chronically exposed to O_3 and a prediction of O_3 dose as a function of distance from the bronchiole-alveolar duct junction (BADJ) to ventilatory units. Using these data, Miller and Conolly (1995) plotted the predicted O_3 dose and the observed change in wall thickness due to the exposure versus distance from the BADJ (Figure 8-14). Even though considerable variability in the thickness change can be inferred from the data, the two curves, scaled to their values at the junction, show a remarkable similarity and suggest a basic correctness in regards to the ventilatory unit model parameters.

8.4.3 Interspecies Delivered Dose Response

The illustrations presented in this section are based on dosimetric estimates for humans and rats using existing or modified theoretical models (Miller et al., 1985; Miller et al., 1988; Overton et al., 1987). One functional and one inflammatory endpoint will be provided drawing from the f and BAL protein data described in Section 8.3. Because the diversity of exposure scenarios across species is so great, the window of exposure parameters has been narrowed, minimizing exposure-based differences in relating species responsiveness.

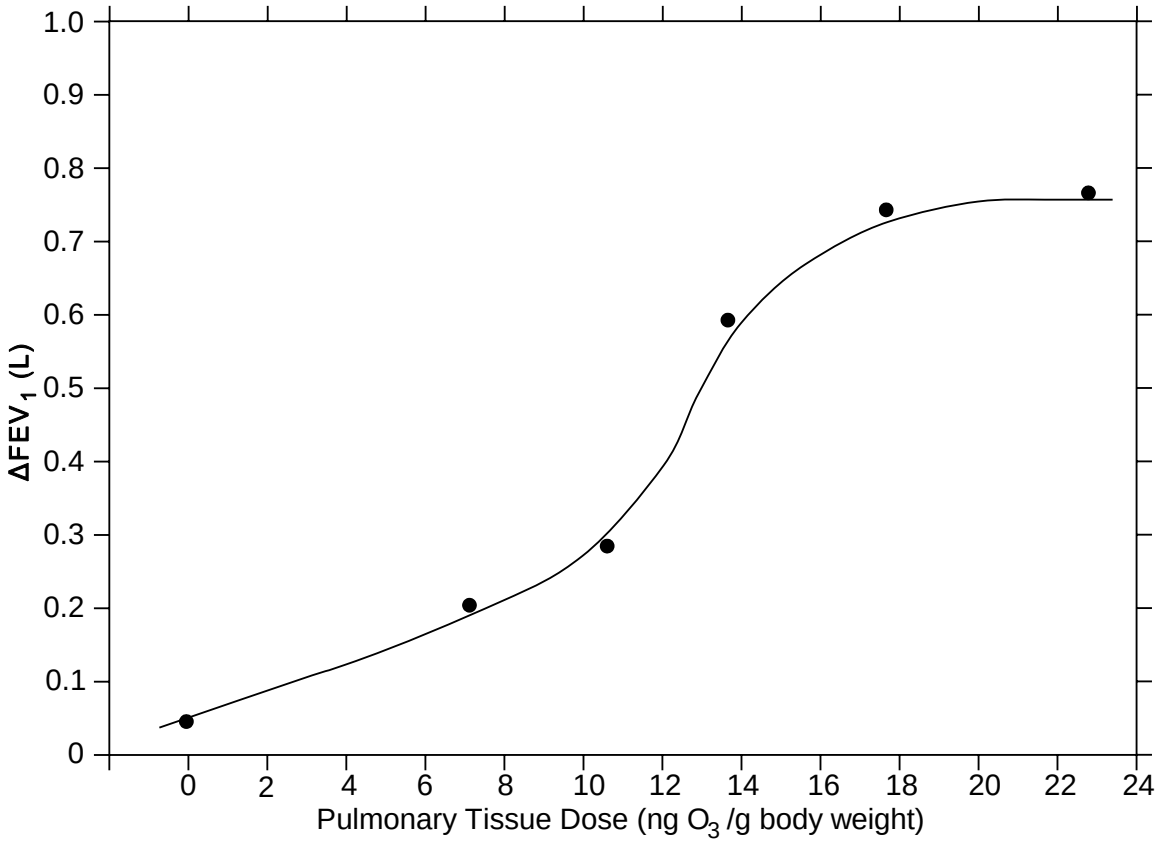


Figure 8-13. Changes in forced expiratory volume in 1 s (ΔFEV_1) versus pulmonary tissue dose. Plotted are decrements in FEV_1 (liters) for human subjects against predicted pulmonary tissue dose normalized to body weight. In order, from left to right, the dose values correspond to 0, 0.12, 0.18, 0.24, 0.30, and 0.40 ppm ozone (O_3) exposure concentrations. The continuous curve was an "eye fit".

Source: Miller et al. (1988)

Because the theoretical models developed by Miller et al. (1985, 1988) and Overton et al. (1987) estimate the dose distribution to the RT on a per breath basis, a minimum quantitative description of tidal breathing (V_T and f) is needed to utilize the theoretical models of O_3 deposition. The need for detailed breathing parameters, therefore, severely restricts the application of the model to studies providing such data. Unfortunately, breath-by-breath parameters over the course of an exposure normally are not measured or reported in most publications. Two studies each in humans and rats, providing adequate detail over the course of the exposure, allowed the model computations to be performed for this illustration (Figure 8-15). The human studies examined were DeLucia and Adams (1977), who exposed the subjects to 0.15 and 0.30 ppm O_3 for 1 h with continuous exercise (65% oxygen uptake to the body) periods, and Beckett et al. (1985), who exposed the

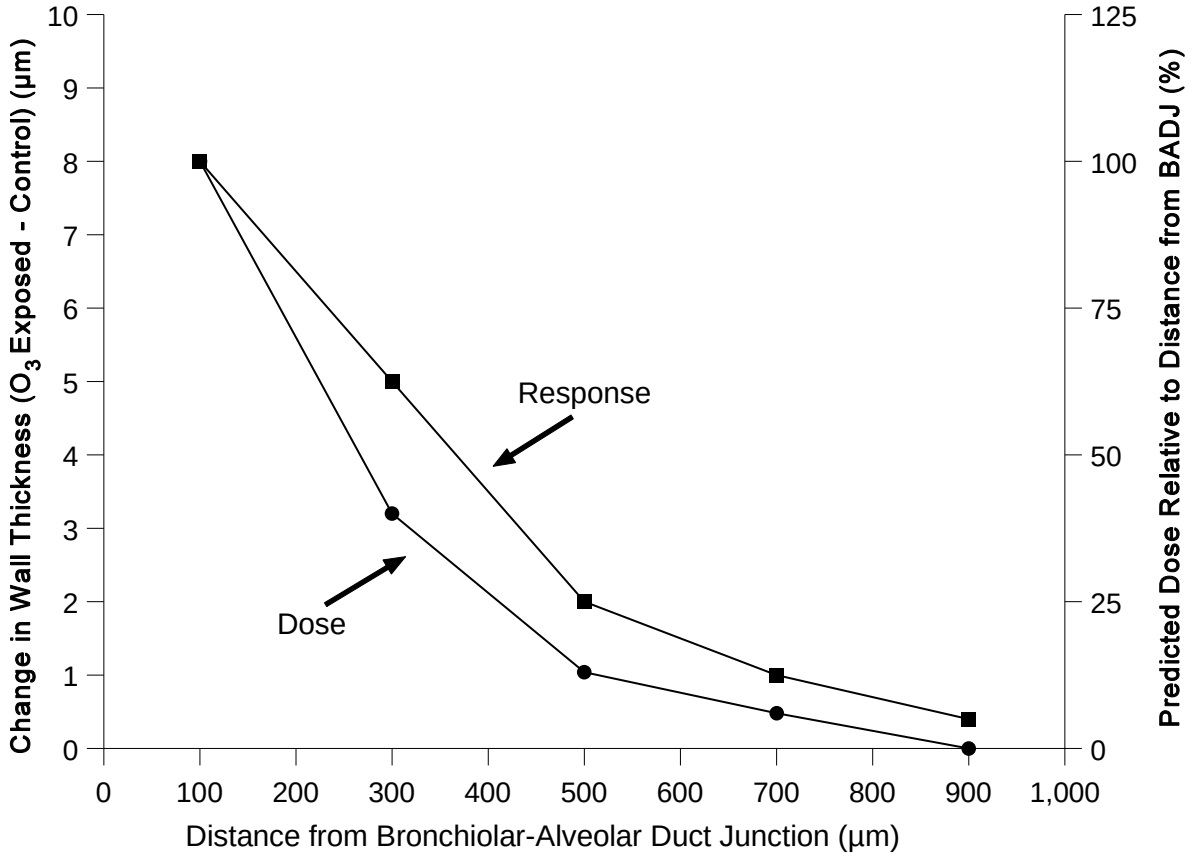


Figure 8-14. Relationship between change in alveolar wall thickness and predicted ozone (O_3) dose as a function of distance from the bronchiole-alveolar duct junction (BADJ). Rats were exposed to 0.98 ppm O_3 for 8 h/day for 90 days.

Source: Miller and Conolly (1995).

subjects to 0.4 ppm O_3 with alternating 15 min exercise (70 L/min). Three rat studies were evaluated: (1) Tepper et al. (1989), who exposed rats to 0.35, 0.50, and 1.00 ppm O_3 for 2.25 h with alternating 15-min periods of CO_2-induced hyperventilation (2 to 3 × resting $\dot{V}_E$); (2) Tepper et al. (1990), who exposed rats to 0.12, 0.25, 0.50, and 1.00 ppm O_3 for 2.25 h with alternating 15-min periods of CO_2, such that subsequent periods of CO_2 exposure had higher CO_2 concentrations than previous periods; and (3) Mautz and Bufalino (1989), who exposed rats to 0.8 ppm O_3 for 3 h (at rest). The response parameter was the ratio of O_3-altered f (f_{O_3}) to control f (f_{cnt}). Dose rate was the average dose to the proximal alveolar region (PAR) computed from time 0 to the time of the maximum f_{O_3}/f_{cnt}. The PAR was chosen as the target based on the perception that O_3 acts on deep lung stretch receptors

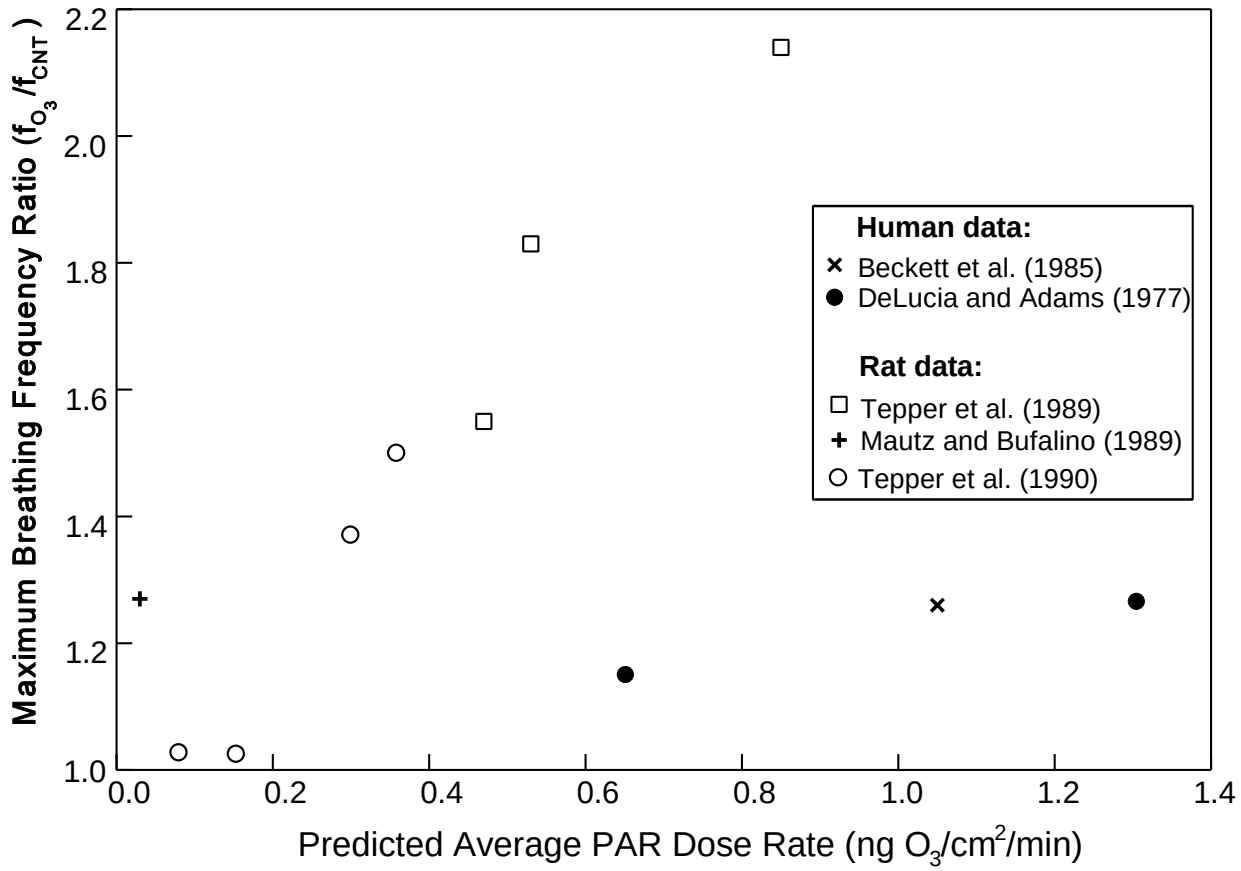


Figure 8-15. The maximum ratio of ozone (O_3)-altered breathing frequency (f_{O_3}) to control breathing frequency (f_{cnt}) at various O_3 concentrations versus predicted average dose rate to the proximal alveolar region (PAR; first generation distal to the terminal bronchioles). The average is for the time from the initiation of exposure to the time of occurrence of the maximum ratio. Beckett et al. (1985) and Mautz and Bufalino (1989) provided sufficient ventilatory data for only one O_3 concentration.

directly or indirectly (via tissue fluid pressure [edema]) as the stimulus for tachypnea. A number of assumptions were necessary to implement the deposition model for comparison of the human and the rat in this context; these are noted in Table 8-6. Although admittedly not validated, Figure 8-15 suggests that the response of the rats not only exceeded that of humans, but was initiated at a lower dose rate to the targeted lung region. This conclusion is in general agreement with that emanating from Figure 8-7, but the dose-to-target approach appears to accentuate the apparent difference in sensitivity for this endpoint in favor of the rat.

Using an analysis similar to that applied above for f, Miller et al. (1988) related pulmonary tissue dose (normalized to body weight) to BAL protein from rats, guinea pigs, and rabbits (Hatch et al., 1986) for O_3 concentrations of 0 to 2.0 ppm for 4 h. These values have

been supplemented in Figure 8-16 to include the results of the human study of Koren et al. (1989b). As can be seen in the illustration, there appears to be a log-normal relationship between BAL protein and dose to the pulmonary region, the purported site of plasma leakage to the airspace lumen. This relationship would support the contention that there is a mechanistic consistency in response across species that may exhibit a quantitative sensitivity factor for use in further quantitative interspecies extrapolation. This sensitivity factor is evident from the clustering of data from different animal species.

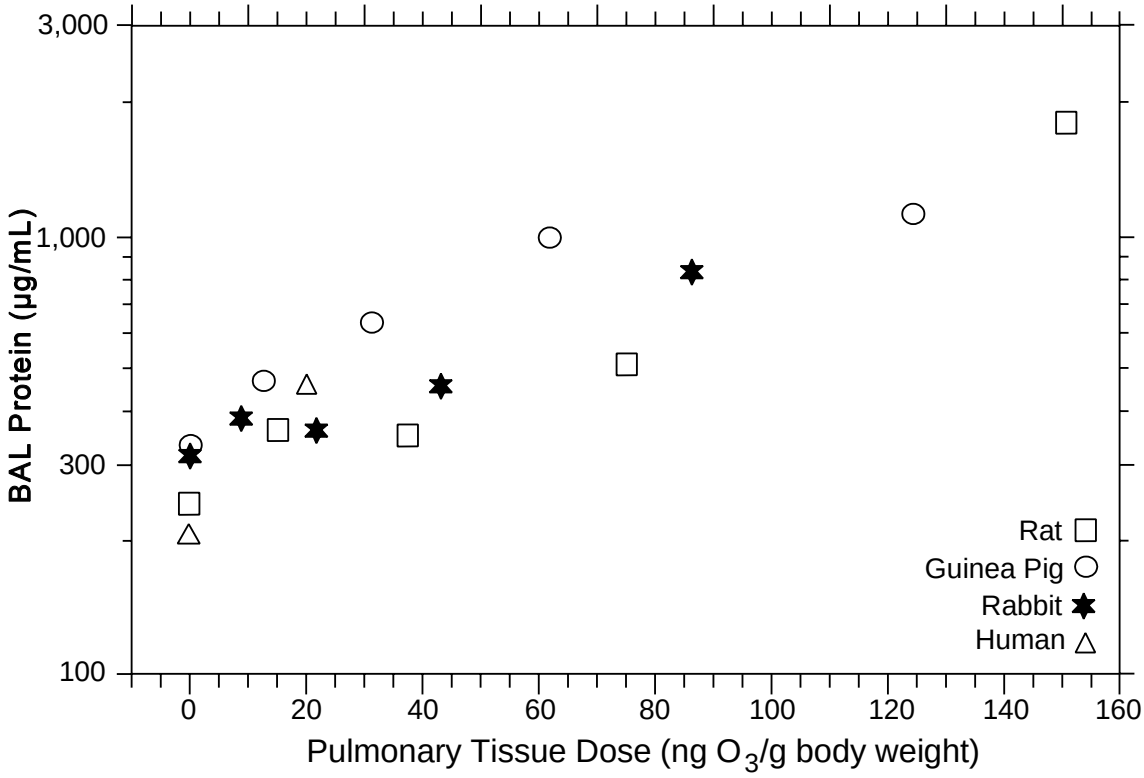


Figure 8-16. Protein in the bronchoalveolar lavage (BAL) for several laboratory animal species and humans, as related to the estimated pulmonary dose (normalized per gram of body weight).

Source: Modified from Miller et al. (1988).

8.5 Quantitative Extrapolation of Chronic Ozone Effects

8.5.1 Introduction

In previous sections, the homology between laboratory animal responses to acute O_3 exposure and effects observed in human subjects was demonstrated. Most of these responses were related to lung function and, to a more limited extent, recovery of extravasated

protein in BAL fluid, because these effects are measurable in both human and nonhuman species. Of laboratory species commonly used to address questions of acute toxicity, rats have the strongest and most compatible database for such comparisons. Studies in nonhuman primates typically are limited to morphology endpoints and to longer exposure periods, thereby limiting the practical utility of these studies in this same context. A conclusion drawn from the discussion of acute effects is that there is reasonable semi-quantitative agreement and homology between species with regard to their functional and permeability responses to short-term O_3 exposure. Where data from long-term exposures exist, cross-species relationships among similar endpoints (usually functional) are considerably weaker, although there is the suggestion that long-term exposure to O_3 can alter the distal lung (that zone where theoretical models would predict greatest O_3 deposition).

This section continues the rationale founded in the extrapolation paradigm illustrated in Figure 8-6 in an attempt to quantitatively address the question of potential chronic alteration to the lungs of O_3-exposed people. Direct data from epidemiological studies remain suggestive at best (see Chapter 7). On the other hand, the extensive database of morphometric effects on the distal lung of exposed animals (rodents and nonhuman primates) reveals the sensitivity of such endpoints and clearly relates to the site of the lung associated with incipient chronic lung diseases associated with known toxic inhalants (e.g., tobacco smoke). Unfortunately, there is a lack of reliable morphometric data on the human lung that are associated with O_3 or other air pollutants. Thus, the goal here is to draw from the extensive and reliable animal database, which demonstrates chronic effects of long-term exposures to O_3, and project from it the potential for chronic effects in humans utilizing the linkage provided by newer, refined O_3 dosimetry estimates.

In the long-term studies selected for detailed analysis, great importance was placed on the relevance of the exposure concentrations, the site specificity of the morphometric analysis, and the consistency of analysis within species. Two rat studies were selected that represent near-lifetime exposures to O_3 over a range of concentrations and scenarios (Chang et al., 1992; Chang et al., 1995). The former study was conducted in conjunction with the U.S. Environmental Protection Agency (EPA) and involved a weekday urban pattern of exposure represented by a 9-h spike (5 days/week) slowly rising to 0.25 ppm from a near-continuous baseline of 0.06 ppm O_3 for 78 weeks. The latter study was conducted by the same morphometrists but on rats exposed weekdays (6 h/day) to 0, 0.12, 0.50, or 1.00 ppm O_3 for 87 weeks. Both studies utilized F344 rats. Likewise, the primate studies were conducted by the same investigators, but involved two strains of monkeys. In one study, the bonnet monkey was exposed for 90 days (8 h/day) to 0, 0.15, or 0.30 ppm O_3 (Harkema et al., 1993), whereas the second study consisted of daily (8 h/day) exposures to the smaller fascicularis monkey of 0 or 0.25 ppm O_3 for 18 consecutive mo or 9 mo with alternating months of clean air (Tyler et al., 1988). The assumptions needed to model the specific species dosimetry of each study are provided in the following section. Growth was compensated where appropriate (for rats), and allometric anatomic adjustments or assumptions were made to estimate unavailable anatomic data (as in the case of the monkey) for the dosimetry models. Ventilation was assumed to be unaffected by O_3 after the first 2 days of exposure because adaptive events are known to occur in that time frame. This simplified the model formulations for the animal studies, although varied activity and exposure profiles were considered throughout for the human dose estimates. Allometric equivalent life-span estimates also were made in an effort to relate the duration of the exposure period relative to life-span and cumulative dose.

In each experiment, a subregion of the CAR (where major lesions have been observed) was chosen by the investigators for study. For the two rat studies, the site was the PAR, which was defined by Chang et al. (1992) as "the alveolar tissue surrounding the alveolar ducts beginning at the bronchiolar-alveolar duct junction and ending at the second alveolar duct bifurcation." For modeling purposes, the first generation distal to the terminal bronchioles corresponded to the PAR. The monkey studies focused on an analogous airway region, the respiratory bronchioles. Thus, in three of the four experiments, the first generation distal to terminal bronchioles was the explicit site of the observed effects. For simplicity, similar assumptions were made for the Tyler et al. (1988) monkey study that came from the same laboratory as Harkema. For discussion purposes, the term PAR has been used for the monkey sites, even though the airway morphology of this site differs between the rat and monkey. The simulated dose of the PAR was chosen for comparison to the reported effect.

Because the PAR generally is thought to be the site of incipient lung disease, it is of particular interest with regard to the potential role of O_3-induced chronic lung disease. Effects in the PAR can be evaluated specifically using morphometric techniques with an electron microscope, and, likewise, dosimetric models can estimate surface-area-normalized focal tissue doses within the same region. The dose-response relationships constructed in this extrapolation focus on the theoretical dose to the PAR of rats and monkeys, with their corresponding impact on the PAR interstitial structure (as total and acellular thickness). Analogous estimated cumulative doses to the PAR of a 9-year-old child and of an outdoor worker exposed to a New York City summer O_3 season then are interpolated from the effects in the experimental animals at nominally similar doses.

In this attempt to extrapolate chronic O_3 effects from the experimental animals to humans, the two foremost assumptions are that (1) there exists coherence of analogous dose-response data across species with regard to acute exposure effects (as represented by one side of the parallelogram model [Figure 8-6]), and (2) there exists some relationship of effect to the total cumulative dose to the target tissue. The essential hypothesis is that the rate of change of interstitial thickness is related to the rate of O_3 uptake. Clearly, the latter assumption ignores potential adaptive or reparative processes, but rather assumes that continuing exposure of any scenario imparts irreversible or slowly reversible changes within the constitutive structure of the target area. There is little to substantiate this assumption other than the commonly believed irreversibility of fibrogenesis and degenerative lung disease.

8.5.2 Factors Considered in Estimating Dose

For each of the animals for which doses were simulated, Table 8-10 lists information or parameters needed for the dosimetry simulations and the source of the information. Also, details are provided as to how this information was modified, scaled, or used to correspond to the experimental or hypothetical animals for which dose was estimated. An expanded discussion follows.

8.5.2.1 Human

The dose simulations were for a hypothetical New York City adult outdoor worker and a hypothetical 9-year-old New York City child, who is active out of doors. Their

Table 8-10. The Basis of Information for Model Parameters[a]

Information Needed	F344 Rat (EPA Chronic Study)	F344 Rat (NTP/HEI Chronic Study)	Bonnet Monkey (*Macaca radiata*)	Monkey (*Macaca fascicularis*)	Human Child	Human Adult
Characteristics of simulated animal	Chang et al. (1992); Tepper et al. (1991): 0.21-0.47 kg, 10-88 weeks old, males.	Chang et al. (1995); Last et al. (1994): 0.13-0.53 kg, 7-93 weeks old, males.	Harkema et al. (1993): 2.3-9.7 kg (used 6-kg), 22-35 weeks old, males and females.	Tyler et al. (1988): 1-2 kg (used 1.6-kg), 30-107 weeks old, males.	Present simulation: 31 kg, 9 years, 132-cm height, male or female.	Present simulation: 73 kg, 181-cm height, adult male.
Exposure regime	Chang et al. (1992); 0.06 ppm continuous background, 22 h/day, 7 days/week; 0.25 ppm ramped peak over 9 h, 5 days/week. 3-, 13-, and 78-week exposures.	Chang et al. (1995); 0, 0.12, 0.50, and 1.00 ppm 6 h/day, 5 days/week for 87 weeks.	Harkema et al. (1993); 8 h/day for 90 days; 0, 0.15, and 0.30 ppm.	Tyler et al. (1988); 8 h/day for 18 mo; 0 ppm, 0.25 ppm (daily), and 0.25 ppm (daily, alternating months).	Johnson (1994); continuous exposure pattern for a New York outdoor child; April-October, 1991. Avg. exposure 22 ppb; range, 0-238 ppb.	Johnson (1994); continuous exposure pattern for a New York outdoor worker. April-October, 1991. Avg. exposure, 23 ppb; range, 0-227 ppb.
LRT structure	PUL: Mercer et al., 1991 (0.283-kg S-D rat). TB: Yeh et al., 1979 (0.33-kg Long-Evans rat).		Weibel (1963): adult human LRT.			
Dead space volume at FRC	Dead space volume = URT + TB volumes.		Dead space volume = URT + TB volumes.		Hart et al. (1963): height: 92-198 cm, age: 4-42 years, weight: 16-115 kg.	
FRC	Mercer et al., 1987 (0.291-kg S-D rat). FRC $\propto$ weight$^{0.55}$ (Takezawa et al., 1980).		Kosch et al., 1979 (radiata monkeys, FRC = 52.8 mL/kg).	Tyler et al., 1988 (2-kg fascicularis). FRC $\propto$ weight$^{0.86}$ (relation for combined rodent species, Takezawa et al. (1980).		
URT volume (V) and surface area (S)	Patra et al., 1987 (F344 rat, 0.012-0.366 kg, 1-63 weeks old). Graphical interpolation and extrapolation with respect to age.		Schreider and Raabe, 1981 (7-kg rhesus monkey). V $\propto$ weight; S $\propto$ V$^{2/3}$.		V: The ratio of URT and dead space volumes are the same as in the adult human. S $\propto$ V$^{2/3}$	Hu et al. (1992a).
TB volume (V) and surface area (S)	V: Mercer et al., 1994a (0.293-kg S-D rat). S: Yeh et al., 1979 (0.33-kg Long-Evans rat). S $\propto$ V$^{2/3}$.		V: Pulmonary and TB volumes in same ratio as human. S $\propto$ V$^{2/3}$.		V: volume = dead space volume minus URT volume. S $\propto$ V$^{2/3}$.	
PUL region volume (V) and surface area (S)	V: FRC minus TB volume. S $\propto$ V$^{2/3}$.		Pulmonary and TB volumes in same ratio as human. S: Mercer et al. (1994b), interspecies interpolation.	V: Pulmonary and TB volumes in same ratio as human. S: Tyler et al., 1988 (2-kg fascicularis). S $\propto$ V$^{2/3}$.	V: volume = FRC volume minus dead space volume. S $\propto$ V$^{2/3}$.	

Table 8-10 (cont'd). The Basis of Information for Model Parameters[a]

Information Needed	F344 Rat (EPA Chronic Study)	F344 Rat (NTP/HEI Chronic Study)	Bonnet Monkey (*Macaca radiata*)	Monkey (*Macaca fascicularis*)	Human Child	Human Adult
Tidal volume and breathing frequency	Costa (1994): measurements made at 1, 3, 13, 52, and 78 weeks of exposure (interpolation for other time points).	None reported. Assumed similar to that of the EPA chronic study.	None reported. A range of parameters used. See Table 8-11 and Section 8.5.2.2.		Johnson (1994): activity pattern. Hofmann et al. (1989): tidal volumes and breathing frequences.	Johnson (1994): activity pattern. ICRP (1975): tidal volumes and breathing frequences.
Mass transfer coefficients	Parameter estimation using the rat data of Hatch et al. (1989) and an assumed pulmonary region coefficient. See Table 8-6 and Section 8.5.2.3.		For corresponding generations or model segments, the same as for the adult human. See Table 8-6 and Section 8.5.2.1.			Hu et al. (1992b); Miller et al. (1985); Weibel (1963). See Table 8-6.

[a]See Appendix A for abbreviations and acronyms. PUL, V, and S indicate pulmonary, volume, and surface area, respectively. In some cells of this table, the information is ordered as follows: characteristics of the species and the reference to the basis of the information, followed by an indication as to how the reference information was used or modified for the present simulations. The proportion symbol $\propto$ indicates that one parameter is proportional to another (e.g., $S \propto V^{2/3}$ implies that $S = S_0 (V/V_0)^{2/3}$, where the subscripted parameters are known; V is the new or desired volume and S is the estimated surface area of the new volume.

residences were not air-conditioned, and they lived, worked, or played in the same area. These two groups of people would have tended to experience higher outdoor O_3 concentrations than other New York City people because the chosen area had higher outdoor O_3 concentrations than other areas, and O_3 levels are higher in homes without air-conditioning (Johnson, 1994). These scenarios were selected because they involve the same at-risk population groups used for the O_3 risk analysis in the staff paper (U.S. Environmental Protection Agency, 1995).

The activity patterns and O_3 exposure estimates were generated by the probabilistic National Ambient Air Quality Standards Exposure Model for O_3 (pNEM/O_3) (Johnson, 1994) for April through October 1991. The modeling approach for outdoor workers can be described as follows. Outdoor workers residing in New York City were divided into nonoverlapping sets of cohorts, such that each cohort could be identified by a residential location, a work location, and a residential air conditioning system. The pNEM/O_3 model generated an "exposure event sequence" (EES) for each cohort based on data obtained from activity diary studies involving outdoor workers. Each exposure event in the EES assigned the cohort to a geographic location, a microenvironment, and an exertion level. Algorithms within the pNEM/O_3 model provided estimates of O_3 exposure and equivalent ventilation rate (EVR) for each event that lasted from 1 to 60 min. The EVR is ventilation rate (liters per minute) divided by body surface area (square meters). Exposures were calculated on a minute-by-minute basis; however, the concentration and EVR values of all minutes within a given exposure event were held constant during the event. The pNEM/O_3 model used for outdoor children was generally consistent with the model for outdoor workers but relied on human activity data from children. Another difference was that the cohorts of children were identified by residential location and air conditioning system only; workplace location was not specified.

Figures 8-17A and 8-17B give an idea of the exposure concentrations for the outdoor worker and child, respectively, with plots of the average hourly and monthly O_3 concentrations. The exposure concentrations of the adult and child differ because their daily activities and locations are different. Note that in a few cases, the hourly average exceeds the 0.12 ppm (120 ppb) standard.

Anatomical Aspects

The adult New York City worker was assumed to have characteristics similar to the subjects of an investigation by McDonnell et al. (1983). The body surface area of the worker (needed to convert EVR to minute volume) was assumed to be 2 m^2 (Koren et al.,1989b 1.90 and 1.98 m^2; Johnson, 1994: 1.90 to 1.95 m^2). The New York City child had a body surface area of 1.07 m^2, which was consistent with both 9-year-old males and females (Johnson, 1994). The assumed values for the child's height (132 cm) and weight (31 kg) were based on Phalen et al. (1985) and Johnson (1994).

For the simulations, no distinction was made between mouth and nose breathing with respect to URT uptake. The significance of this assumption is not clear. If both modes have approximately the same uptake efficiency, then the assumption of no distinction between mouth and nose breathing is appropriate for predicting PAR doses. Whether this is a valid assumption has not been settled (see Section 8.2.3.4). The same URT anatomical model was used for both ages, but was isotropically scaled as necessary. Surface areas and lengths were assumed to be proportional to the volume to the two-thirds and one-third powers for adults and children, respectively (e.g., if x is proportional to volume V to the power p, then

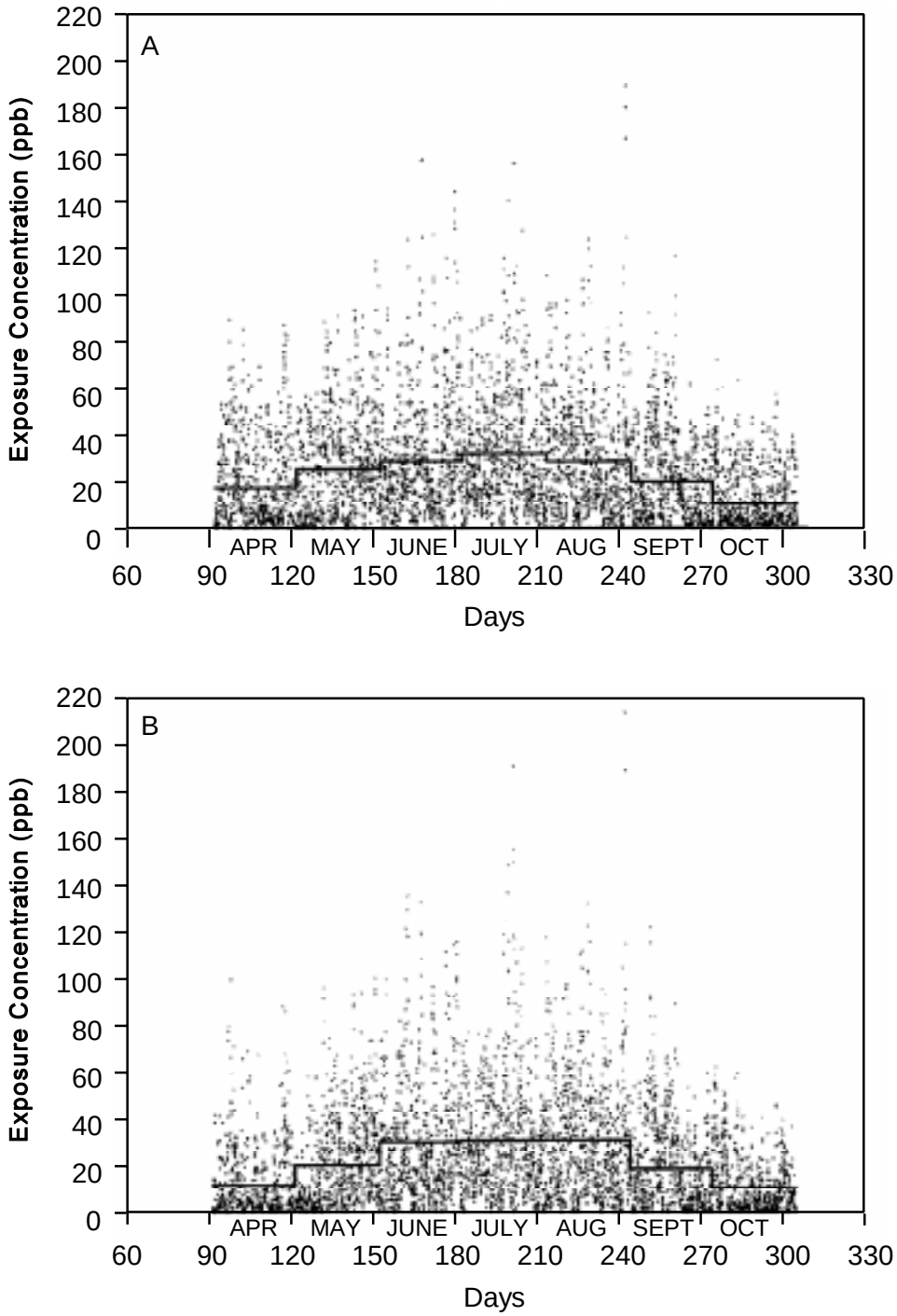


Figure 8-17. The variation in exposure concentration for the New York City adult and child. Plotted are the hourly and monthly O_3 averages for the New York City outdoor worker (A) and the 9-year-old child (B). The hourly averages are represented by the "dots" and the monthly average by the continuous solid line.

$x_n = x_0(V_n/V_0)^p$, where the subscripts n and o indicate the new and original values, respectively). Dimensions used for the adult were from Hu et al. (1992b), who based their values on the acoustic reflection measurements of Fredberg et al. (1980) that corresponded to the oropharyngeal (mouth through larynx) region. The LRT model developed by Weibel (1963) was used for LRT structure and dimensions, but was scaled isotropically to FRC and dead-space volumes, as appropriate.

Mass Transfer Coefficients

This parameter is necessary to determine the local dose, expressed in terms of the flux of O_3 to respiratory tract surfaces. Specifically, this dose is related to the product of the mass transfer coefficient and gas-phase concentration of O_3 integrated over the time of interest. Good estimates of mass transfer coefficients are needed to predict a reliable dose at specific respiratory tract sites.

A brief description of how the human URT and TB region mass transfer coefficients were derived from Hu et al. (1992b) and Weibel (1963) is presented in Table 8-6. These coefficients were "validated" by simulating the bolus-response experiment of Hu et al. (1992b). Simulation results of the bolus uptakes overpredicted by □5%; results for the other two measured variables deviated from the experimental values by □30%.

As a result, simulations with inhaled and exhaled flow rates of □250 mL/s are expected to overpredict total uptake by no more than 5%. The significance of the poor results in simulating the other two variables is not clear. Possibly, predictions of the distribution of absorbed O_3 within the RT are questionable; also, predicted total uptakes for flow rates different from the experimental value of 250 mL/s may be suspect. For very different reasons, this latter speculation is probably valid. Measurements by Hu et al. (1994) at rates higher than 250 mL/s indicate that local mass transfer coefficients increase with increasing flow rates. In addition, naive subjects were used for the bolus experiments. Chronic exposure to O_3, which is more appropriate to the present simulation scenarios, could alter mass transfer coefficients due to chemical reactions changing the properties of biochemical constituents. See Section 8.2.4.2 and Table 8-7 for a comparison of dosimetry modeling results with dosimetry data, using coefficients based on Hu et al. (1992b).

The experimental data of Hu et al. (1992b) were not sensitive enough to allow an estimate, with confidence, for the pulmonary region or for the PAR mass transfer coefficient. For this reason, these coefficients were assumed to be the same as used by Miller et al. (1985). The value of this parameter is approximately the same as used for the rat. Although the value of the pulmonary region mass transfer coefficient of rats also is unknown, a comparison by Pinkerton et al. (1992) of morphometric effects and dosimetry model predictions suggests that the rat value is not unreasonable.

Ventilation

Activity patterns for the adult and the child (Johnson, 1994) were used to estimate $\dot{V}_E$s. The set of estimates had over 300,000 minute-by-minute cases, each case consisting of the day of the year, hour, minute, and equivalent ventilatory rate ($\dot{V}_E$ divided by body surface area). The generated patterns were for 214 sequential days, from the beginning of April through October 1991.

For the adult, V_Ts were estimated by interpolation from a plot of V_T versus $\dot{V}_E$ that was drawn using information from Table 120 in International Commission on Radiological

Protection (1975). Tidal volumes and f's for the child were estimated using a relationship between V_T and $\dot{V}_E$ developed by Hofmann et al. (1989). For both humans, f's were defined as $\dot{V}_E$ divided by the estimated V_T.

Estimating Proximal Alveolar Region Dose

The PAR doses were estimated by simulation for each of the minute-by-minute equivalent $\dot{V}_E$s and personal concentration estimates; these doses were averaged.

8.5.2.2 Monkeys

Anatomical Aspects

Because there were no data on the uptake of O_3 by the URT of monkeys, no distinction was made between oral and nasal breathing. The URT dimensions were based on Schreider and Raabe (1981) for the nasopharyngeal region of a 7-kg rhesus monkey. Volumes were proportional to body weight and dimensions were scaled isotropically. For the nasopharyngeal region, the dimensions used may have been too large because the casting procedure may have resulted in a larger volume (Yeh et al., 1989) than in a live animal, and air flow streamlining (Morgan et al., 1991) may have resulted in a smaller effective volume. The structure of the LRT was based on Weibel (1963) for humans (the same as used for the human simulations), which was isotropically scaled to monkey FRC values, assuming that TB and pulmonary region volumes were in the same proportion as the human FRC values. The use of the human LRT structure for the monkey simulations is considered to be of less importance to reasonable predictions than the correct specification of volumes and surface areas of the different regions.

Mass Transfer Coefficients

The human mass transfer coefficient values were assumed because there were no reported values nor uptake data that would have allowed estimates of these coefficients. For corresponding model segments or model generations, the monkey coefficients were the same as those used for humans.

Ventilation

No ventilation parameters were reported for the monkey experiments. For this reason, four states of ventilation were used to calculate the doses (see Table 8-11). The first three in the table were extrapolated from human values of V_Ts and f's. These human parameters were consistent with the algorithm used for the human simulations and correspond approximately to sedentary, low, and light activity as categorized by Hofmann et al. (1989) for an adult human. The last set of parameters correspond to measurements made by Moss (1995) on five awake female adult cynomolgus monkeys (*Macaca fascicularis)*.

Estimating Proximal Alveolar Region Doses

For the two monkey experiments and corresponding ventilatory parameters, scaled PAR doses were estimated and multiplied by the average exposure concentrations to obtain estimated average PAR doses.

8.5.2.3 Rats

Anatomical Aspects

Rats are nasal breathers; therefore, the URT model corresponds to and is based on

Table 8-11. Estimating Monkey Ventilatory Parameters[a]

Human Activity Level[b]	Extrapolation from a 73-kg Human with the Following Parameters[c]			To a 1.6-kg Monkey			To a 6-kg Monkey		
	$\dot{V}_E$ (L/min)	V_T (mL)	f (breaths/min)	$\dot{V}_E$ (L/min)	V_T (mL)	f (breaths/min)	$\dot{V}_E$ (L/min)	V_T (mL)	f (breaths/min)
Sedentary	6	500	12.0	0.34	11.0	31.2	0.92	41.1	22.4
Low	12	900	13.3	0.68	19.7	34.7	1.84	74.0	24.9
Light	20	1,300	15.4	1.14	28.5	40.0	3.07	107.0	28.7
	Extrapolation from a 4.4-kg *Macaca fascicularis* with the following parameters[d]								
	1.78	52.9	33.6	0.83	19.3	43.2	2.25	72.3	31.1

[a]See Appendix A for abbreviations and acronyms. For extrapolation: $(V_T)_1 = (V_T)_2 \times (\text{weight}_1/\text{weight}_2)$; $f_1 = f_2 \times (\text{weight}_1/\text{weight}_2)^{0.25}$; $\dot{V}_E = V_T \times f$. Subscript 1 corresponds to the 1.6- or 6-kg monkey, and subscript 2 corresponds to either the human or the 4.4-kg monkey.

[b]These characterizations for the human parameters are consistent with those of the International Commission on Radiological Protection (1975) and Hofmann et al. (1989).

[c]For a given human $\dot{V}_E$, the human V_T and f are based on the International Commission on Radiological Protection (1975). For the selected $\dot{V}_E$, V_T and f are the same as used for the adult human.

[d]Moss (1995); parameters are the average for five monkeys.

the F344 rat nasopharyngeal region. The dimensions may be too large, however, because

the casting procedure can result in a larger volume than in a live animal (Yeh et al., 1989), and air flow streamlining (Morgan et al., 1991) may result in a smaller effective volume. The structure of the LRT is a composite of the TB model of the Long-Evans rat (Yeh et al., 1979) and a pulmonary region based on the Mercer et al. (1991) ventilatory unit model of the S-D rat. Given the dosimetry model used, the volumes and surface areas of the different regions are more important than the structural differences of the three strains used to construct the rat RT.

Mass Transfer Coefficients

Mass transfer coefficients for the nasopharyngeal region and TB of the rat were estimated using the uptake data of Hatch et al. (1989) and an assumed pulmonary region mass transfer coefficient. Hatch et al. (1989) reported the average uptake of O_3 for eight F344 rats and the average fraction of $^{18}O_3$ in the nasopharyngeal region, the trachea, and the lungs. (Hatch et al. [1989 and 1994] discuss issues related to using $^{18}O_3$ dose as a measure of O_3 dose.) Based on a discussion in Miller et al. (1993) concerning the investigation of Pinkerton et al. (1992), the pulmonary region mass transfer coefficient was defined as that used by Mercer et al. (1991). This, combined with information from Hatch et al. (1989), allowed an estimate of mass transfer coefficients for the nasopharyngeal region and the TB. A common estimate of mass transfer coefficients also was made using the data for the individual rats. For these coefficients, whose values were essentially the same as the first set, the individual rat simulations deviated from the experimental data by an average of 23%.

Ventilation

For the EPA chronic study, V_T and f were measured at Exposure Weeks 1, 3, 13, 52, and 78. For intermediate weeks, these parameters were estimated by interpolation and assumed constant during each of those weeks. Because ventilatory parameters were not reported for the National Toxicology Program/Health Effects Institite (NTP/HEI) chronic study, parameters similar to those of the EPA chronic study were assumed (see below).

Estimating Proximal Alveolar Region Doses

For the EPA chronic study, the exposure pattern was variable during the week, but repeated each week (Tepper et al., 1991). The PAR doses were simulated for each week and averaged. The ventilatory, physiological, and anatomical characteristics of the NTP/HEI study rats with respect to time were assumed to be similar to the EPA chronic study rats, and the average scaled PAR dose ([g/cm^2-min]/[g/m^3 ambient O_3]) was defined as the same that was predicted for the EPA study rats. Given this and the average exposure concentration, the average PAR doses were estimated.

8.5.3 Results and Discussion

8.5.3.1 Simulation Results

The simulation results are presented in Table 8-12. The first and second columns identify the laboratory experiments and the hypothetical human exposures that were simulated. Column 4 applies only to the monkeys and is discussed above (see Section 8.5.2.2) in the discussion of monkey ventilation. The "Average PAR Dose" (column 5) is the predicted average flux of O_3 to the surfaces of the alveoli or respiratory

Table 8-12. Summary of Simulation Results[a]

Simulation of	Source for Simulation	Average Weight (kg)	Minute Volume[b] (L/min)	Average PAR Dose (μg/cm²-min)	Cumulative PAR Dose (μg/cm²)	Duration of Experiment (weeks)	Equivalent Human Child Time[c] (weeks)	Equivalent Human Adult Time[c] (weeks)
EPA rat (3 weeks)	Chang et al. (1992) Tepper et al.(1991)	0.24		4.11e-5	1.24	3	8.9	11
EPA rat (13 weeks)		0.29		3.85e-5	5.1	13	39	48
EPA rat (78 weeks)		0.4		3.43e-5	27	78	232	286
NTP/HEI rat (0.12 ppm)[d]	Chang et al. (1995)	0.4		0.828e-5	7.3	87	259	319
NTP/HEI rat (0.5 ppm)[d]		0.4		3.45e-5	30.3	87	259	319
NTP/HEI rat (1 ppm)[d]		0.4		6.89e-5	60.5	87	259	319
Bonnet monkey (0.15 ppm)	Harkema et al. (1993)	6		1/2 below	[e] 1/2 below[e]	12.9	19.5	24
Bonnet monkey (0.3 ppm)		6	0.92	0.61e-5	0.79	12.9	19.5	24
			1.84	3.99e-5	5.17	12.9	19.5	24
			3.07	9.92e-5	12	12.9	19.5	24
			2.25	5.65e-5	7.3	12.9	19.5	24
Fascicularis monkey (daily; 0.25 ppm)	Tyler et al. (1988)	1.6		2 × below	2 × below[f]	78	[f] 164	203
Fascicularis monkey ("seasonal"; 0.25 ppm)		1.6	0.34	0.197e-5	1.55	78	164	203
			0.68	1.26e-5	9.91	78	164	203
			1.14	3.25e-5	25.6	78	164	203
			0.83	1.81e-5	14.3	78	164	203
9-year-old NYC[g] child	U.S. Environmental Protection Agency (1995)	31		3.2e-5	9.9	30	30	NA
Adult NYC[g] outdoor supervisor		73		2.78e-5	8.6	30	NA	30

[a]See Appendix A for abbreviations and acronyms.
[b]Only relevant for the monkeys. See Table 8-11.
[c]Given the nonhuman exposure time, the number of weeks the human would have to be exposed for equal human and nonhuman physiological times: $weeks_{HUMAN} = weeks_{NONHUMAN} \times (weight_{HUMAN}/weight_{NONHUMAN})^{0.25}$.
[d]The relative PAR dose [(g/cm²-min)/(g/m³ O_3)] was assumed to be the same as the average for the EPA rat.
[e]For each minute volume, the exposure concentration for these bonnet monkeys was one-half those listed below.
[f]For each minute volume, the fascicularis monkeys exposed "daily" were exposed to O_3 twice as long as the "seasonal" fascicularis monkeys.
[g]NYC = New York City.

bronchioles in the first model generation distal to the terminal bronchioles. For each experiment, the average is over the total time or duration of the experiment (given in column 7, "Duration"); the average includes times of no exposure. Column 6, "Cumulative PAR Dose," is the total quantity of O_3 predicted to be absorbed by the tissue and liquids of the PAR during the experiment; these dose values are the same as the duration (in minutes) multiplied by the average PAR dose (column 5).

The entries in columns 8 and 9 are the total times a human would have to be exposed to reach the equivalent physiologic times of a specific laboratory animal species. Basically, the concept of physiological time is that the time courses of equivalent processes (e.g., a breath, a heartbeat) across species are approximately the same with respect to the time span of the process divided by the body mass raised to the one-fourth power (Travis et al., 1990). If this concept is applicable to the time course of O_3-induced biological effects, then the entries in the two columns are the human exposure durations necessary to obtain the same cumulative PAR dose biological effect as that of the corresponding laboratory animal duration. Note that for only two or three of the laboratory animal experiments are the human durations greater than the laboratory animals' equivalent or real (duration) times.

8.5.3.2 Interpretation of Chronic Site-Specific Dose-Effect Estimates

Because the PAR is considered the primary site of O_3 injury and represents that region of the lung from which most chronic lung diseases originate, it was selected as the most appropriate target to develop cross-species dose-response extrapolations. The selected "effect" relates to the thickness of the interstitium at the PAR, which is indicative of fundamental structural remodeling. The indices of thickness provided in the noted studies include both cellular and acellular constituents, a distinction that was not always clear; however, interstitial arithmetic thickness, areal volume (cubic micrograms per square microgram), or volume density was available and could be represented in terms of percent change from control.

Dose-response curves for O_3-induced thickening of the PAR are represented in Figures 8-18A (rats) and 8-18B (monkeys). The cumulative PAR dose for the specific exposure scenario (from Table 8-12; Cumulative PAR Dose) for each study is provided on the abscissa as is its corresponding percent change in total and acellular interstitial (PAR) thickness on the ordinate (detailed in Table 8-13). The rat studies are plotted separately from the monkey studies because of an approximate three- to fivefold difference in their responses, with the monkey being more responsive than the rat. Although this difference could represent innate sensitivity differences between the species, it should be noted that estimates of daytime rat exposures (a period of quiescence for the rat), in contrast to the daytime-active monkeys, may have been substantially lower in terms of dose than that predicated on the basis of rat ventilatory measurements that were derived when they were in an aroused, awakened state. Recent studies comparing rat and human $^{18}O_3$ dosimetry indicate that exercise can account for up to a fivefold difference in acute responsiveness between species (Hatch et al., 1994). These explanations remain speculative, however, in the absence of direct data. Ventilation values for the monkeys were selected a priori as light activity to be similar to the human model being used, which had ventilation values only slightly higher than the empirically derived values (see Table 8-11) from comparable awake, resting monkeys (when adjusted for size).

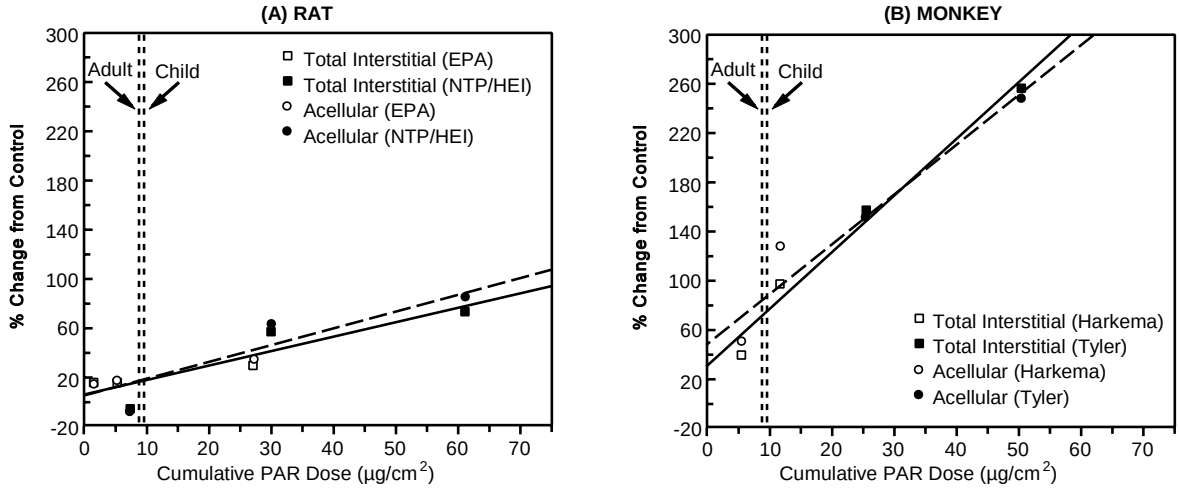


Figure 8-18. (A) Change from control of total interstitial and acellular thickness for the rats exposed to ozone (O_3) in the U.S. Environmental Protection Agency (Chang et al., 1992) and National Toxicology Program/Health Effects Institite (Chang et al., 1995) studies. Solid line represents the linear regression for the total interstitial thickness, and the dashed line represents the linear regression for the acellular thickness across the various cumulative dose estimates for both studies. The respective correlation coefficients (r^2) are 0.84 and 0.80. (B) Change from control of total interstitial and acellular thickness for the monkeys exposed to O_3 in the Tyler et al. (1988) and Harkema et al. (1993) studies. Solid line represents the linear regression for the total interstitial thickness, and the dashed line represents the linear regression for the acellular thickness across the various cumulative dose estimates for both studies. The respective correlation coefficients (r^2) are 0.97 and 0.93. Vertical dashed line with arrows for child and adult denote proximal alveolar region (PAR) dose level for interpolation of PAR thickness effect from the monkey upper estimate of response.

What is remarkable in both Figures 8-18A and 8-18B is the apparent linear dose-response relationships within species. This may be due, in part, to the fact that there was consistency in the methods of the respective investigatory team studying each species. It is interesting to note that the estimated total cumulative PAR dose for the EPA chronic study (27 μg O_3/cm^2) is quite similar to that estimated for the NTP/HEI study group of 0.5 ppm O_3 (30.3 μg O_3/cm^2). An examination of the respective PAR thickness response from each study indicates an apparent similarity between the studies, with the NTP/HEI results being a bit larger in value. At 0.5 ppm O_3, the NTP/HEI investigators also reported the observation of bronchiolarization into the PAR, whereas this process was absent or not significant at 0.12 ppm O_3 or in the EPA chronic study. It is not clear whether this indicates qualitatively, rather

than quantitatively, different responses initiated at 0.12 and 0.50 ppm O_3. However, it is important to note that the correlation coefficients (r^2) for PAR dose to response across

Table 8-13. Summaries of Study Data Used in Extrapolation of Chronic Ozone Effects[a]

Study Species	Total Interstitial Volume (mean value — $\mu m^3/\mu m^2$)	Cellular Volume ($\mu m^3/\mu m^2$)	Acellular Volume ($\mu m^3/\mu m^2$)
F344 Male Rat; Chang et al., 1992 (EPA)—78 weeks (urban profile)			
• Profile: 0.06-ppm continuous background, 22 h/day, 7 days/week; 0.25-ppm ramped peak over 9 h (avg. 0.19 ppm for peak; 0.09 ppm daily), 5 days/week			
• 3-week exposure (from Figure 4)	(+16% estimate from figure)	(+12%)	(+15%)
• 13-week exposure (from Table 4)	0.398[b] 0.462 (+16%)[c]	0.153[b] 0.174 (+14%)	0.245[b] 0.288 (+18%)
• 78-week exposure (from Table 4)	0.473[b] 0.619 (+31%)	0.149[b] 0.179 (+20%)	0.325[b] 0.440 (+35%)
• 16-week recovery after 78 weeks air • 16-week recovery after 78 weeks O_3	0.531[b] 0.580 (+9%)	0.171[b] 0.168 (0%)	0.360[b] 0.410 (+14%)
F344 Male Rat; Chang et al., 1995 (NTP/HEI)—87 weeks (6h/day, 5 days/week)	From Appendix D1—HEI Report		
• 0 ppm	0.520	0.161	0.360
• 0.12 ppm	0.497 (−5%)	0.162 (0%)	0.335 (−7%)
• 0.50 ppm	0.826 (+58%)	0.239 (+48%)	0.587 (+63%)
• 1.00 ppm	0.902 (+73%)	0.233 (+45%)	0.669 (+85%)
Fascicularis Monkey; Tyler et al., 1988—18 mo (8 h/day)	Volume fraction of lung occupied by respiratory bronchiolar wall ($\times 10^{-3}$)—from Table II		
• 0 ppm	0.716	0.110	0.606
• 0.25 ppm (daily)	2.550 (+256%)	0.437 (+297%)	2.113 (+248%)
• 0.25 ppm (daily; alternating months)	1.830 (+156%)	0.297 (+170%)	1.533 (+152%)
Bonnet Monkey; Harkema et al., 1993—90 days (8 h/day)	Arithmetic mean thickness (μm)—from Figure 3 and text		
• 0 ppm	6.5	3.2	3.3
• 0.15 ppm	9.2 (+41%)	4.2 (+30%)	5.0 (+51%)
• 0.30 ppm	13.1 (+102%)	5.6 (+74%)	7.5 (+128%)

[a]See Appendix A for abbreviations and acronyms.
[b]Control value.
[c]Parenthetical values indicate percent difference from respective control.

both studies as illustrated in Figure 8-18A are 0.80 and 0.84 for the total and acelluar interstitial changes, respectively. In the case of the monkey data represented in Figure 8-17B, the analogous correlation coefficients (r^2) were 0.97 and 0.93 for the total and acellular interstitial effects, respectively. These highly significant correlations suggest that interstitial injury is indeed a cumulative process throughout the exposure history. Moreover,in the case of monkeys with seasonal (intermittent monthly) exposures, there was no loss of effect (i.e., reversal) during the air periods. The response was strictly a function of cumulative dose.

The availability of exposure/activity data for a 9-year-old child and adult outdoor worker over an "exposure-season" of 214 days in New York City provides the opportunity to estimate an analogous PAR dose in these human individuals for comparison to the animal dose-response relationships. Because the rat exposure scenario extends over the majority of a lifetime, an attempt was made to determine the equivalent human exposure time using standard, accepted algorithms for lifetime transformations (see Table 8-12). However, the extrapolation of human biologic exposure time from that of the rat did not yield reasonable results. The primate-based extrapolation of exposure-time fared slightly better but also was less than adequate for the purposes of this exercise. Estimates of accumulated dose, however, were in general agreement (see Table 8-12) and served as the basis for the cross-species extrapolation of effects described below.

Unfortunately, there are no hard data to substantiate whether the rat or monkey better represents the human in the context of the endpoint being addressed herein. The monkey could be favored on the basis of responsiveness at low levels of O_3, corresponding to those in human spirometric tests following exposures involving exercise; however, the dose-adjusted BAL protein data are comparable for humans and rodents. The apparent differences in sensitivity between the rat and monkey may, on the other hand, reflect more rapid repair in the rat than in the primate. This concept would be consistent with the algorithms of Travis et al. (1990), which are based on intrinsic metabolic rates and thus may reasonably apply to repair processes after injury or damage from oxidant-lung surface interactions. Thus, the responses for the rat and monkey may best be considered as bracketing the human response. The linearity of the dose-response relationships in both the rat and monkey models lends credibility to interpolation (Figures 8-18A and 8-18B) of the estimated dose to the human PAR to its corresponding response. For example, in the case of the child, the predicted seasonal response could range from about a 20 to 75% increase in PAR thickness; the adult human response would be slightly less with a 15 to 70% increase. Although the actual changes in the human may not be as large as the monkey, the graphical data from both species suggest that recovery may not be complete (in fact, there was no reversal) when exposure is interrupted during alternating months. Likewise, in rats exposed long-term, reversal was incomplete (66%; see Table 8-13) after a 4-mo postexposure period in clean air. In the case of the rat, 4 mo represents a larger proportion of its life span than a similar period in either the monkey or human, thereby suggesting that even 6 to 8 mo of background "off season" levels of ozone would not be sufficient for complete recovery in primates.

This attempt to extrapolate results of animal studies for the chronic effects of O_3 to the human obviously must be considered preliminary because of lingering questions regarding relative dosimetry across species and the uncertainties associated with episodic (typical human scenario) versus continual, repeated exposures (typical of animal studies). Yet, this extrapolation provides a foundation from which additional questions can be derived and addressed to reduce these uncertainties. The coherent evidence in hand suggests that there is a

real possibility that chronic exposure to O_3 can lead to interstitial thickening at the PAR, that region of the lung involved in chronic diseases such as chronic bronchiolitis associated with cigarette smoke or occupational fibrogenesis. The apparent lack of reversal of effects during periods of clean air raises concern that seasonal exposures have a cumulative impact over many years. The role of adaptive processes in this response is unknown but may be critically dependent on the temporal frequency or profile of exposure. Furthermore, the interspecies diversity in apparent sensitivity to the chronic effects of O_3 is notable, but the issue of dosimetry may be explanatory, in part. It would appear that the rat probably represents no less than the lower limit of response and the monkey the upper limit, if not a direct 1:1 correlate as could be speculated on the basis of relative equivalent lifetime estimates and their phylogenetic relationship.

8.6 Summary and Conclusions

8.6.1 Ozone Dosimetry

There have been significant advances in O_3 dosimetry since publication of the previous O_3 criteria document (U.S. Environmental Protection Agency, 1986) that better enable quantitative extrapolation with marked reductions in uncertainty. Prior to 1986, there were limited data on O_3 uptake in laboratory animals (Yokoyama and Frank, 1972; Miller et al., 1979), essentially no reliable data in humans (Clamann and Bancroft, 1959; Hallett, 1965), only one realistic model of O_3 dose (Miller et al., 1978, 1985), and no data on O_3 reaction kinetics in lung lining fluids. At the present time, data gaps in all of these areas have begun to fill in. Experiments and models describing the uptake efficiency and delivered dose of O_3 in the RT of animals and humans are beginning to present a clearer picture than previously has existed.

The total RT uptake efficiency of rats at rest is approximately 0.50 (Wiester et al., 1987, 1988; Hatch et al., 1989). Data from excised rat lungs support these in vivo findings, and further indicate that O_3 uptake efficiency is chemical reaction dependent (Postlethwait et al., 1994). Of the O_3 taken up by the total RT of the rat, 0.50 is removed in the head, 0.07 in the larynx/trachea, and 0.43 in the lungs (Hatch et al., 1989). The regional uptake efficiency data from the rat have been useful in estimating O_3 mass transfer coefficients for the rat.

Ozone dosimetry models require input of regional mass transfer coefficients. Limited studies have been conducted to quantitate the mass transfer coefficients of lung tissue directly using excised animal tissue. In pig and sheep tracheae, mass transfer coefficients were determined for unidirectional flow conditions and were found to be independent of flow, suggesting a lack of dependence of O_3 uptake on gas-diffusion processes (Ben-Jebria et al., 1991). These findings contrast with Aharonson et al. (1974), who found that the mass transfer coefficient in dog NP increased as a function of increasing flow.

In humans at rest, the total RT uptake efficiency is between 0.80 and 0.95 (Gerrity et al., 1988; Hu et al., 1992b; Wiester et al., 1996). At V_Ts around 500 mL, total RT uptake efficiency falls from about 0.9 to 0.75 as flow increases from 250 to 1,000 mL/s. As V_T increases, uptake efficiency increases and flow dependence lessens, suggesting that, at high V_T, uptake may be gas diffusion limited. At a V_T around 1,500 mL, total RT uptake falls from 0.96 at a flow of 250 mL/s to 0.92 at a flow of 1,000 mL/s. The studies of Gerrity et al. (1988) and Wiester et al. (1996) indicate that the mode of breathing (oral versus nasal versus

oronasal) has little effect on URT or on total RT uptake efficiency. This observation is supported by experiments comparing pulmonary function response as a function of mode of breathing (Adams et al., 1989; Hynes et al., 1988). Kabel et al. (1994), however, found that URT uptake efficiency was lower with mouthpiece breathing as compared with nasal breathing. One possible explanation of the discrepancy among the studies is that a mouthpiece may decrease URT uptake efficiency in comparison with unencumbered breathing. The enhanced physiologic response to O_3 with mouthpiece breathing, shown by Adams et al. (1989), supports this concept.

To obtain data on regional O_3 uptake efficiency in humans, Gerrity et al. (1995) measured O_3 concentrations at various anatomical sites (from the mouth to bronchus intermedius) in spontaneously breathing humans. They found that the unidirectional uptake efficiency of the human trachea was similar to that of the sheep and pig trachea (Ben-Jebria et al., 1991), suggesting a similar mass transfer coefficient behavior in the human trachea. Gerrity et al. (1995) also found that the uptake efficiencies between the mouth and various anatomical sites in the total RT agreed well with the O_3 bolus data of Hu et al. (1992). Both the Hu et al. (1992b) and Gerrity et al. (1995) data indicate that the mass transfer coefficients of the large conducting airways are larger than had been thought previously.

When all of the animal and human in vivo O_3 uptake efficiency data are compared, there is a good degree of consistency across data sets. This agreement raises the level of confidence with which these data sets can be used to support dosimetric model formulations.

In the area of mathematical model formulation, there have been several models developed since 1986. They can be grouped according to how transport and chemical reactions are modeled: instantaneous reactions or quasi-steady, first-order reactions. The models (Overton et al., 1987; Miller et al., 1988; Overton et al., 1989; Hanna et al., 1989; Grotberg et al., 1990) predict that net O_3 dose to lung lining fluid plus tissue gradually decreases distally from the trachea toward the end of the TB, and then rapidly decreases in the pulmonary region. When the theoretical dose of O_3 to lung tissue is computed, it is low in the trachea, increases to a maximum in the terminal bronchioles of the first generation of the pulmonary region, and then decreases rapidly distally into the pulmonary region. The models also provide insight into the role that increased ventilation plays in enhancing O_3-induced responses. The increased V_T and flow, associated with exercise in humans or CO_2-stimulated ventilation increases in rats, shifts O_3 dose further into the periphery of the lung, causing a disproportionate increase in distal lung dose. This prediction is supported by the data of Postlethwait et al. (1994) in excised rat lungs and of Hu et al. (1992b) and Gerrity et al. (1995) in human lungs.

Ozone dosimetry models also have enabled examination of regional dosimetry among parallel and serial anatomical structures. When asymmetric lung morphology is used in dosimetric models, the variation of O_3 dose among anatomically equivalent ventilatory units as a function of path length from the trachea has been predicted to vary as much as sixfold (Overton et al., 1989; Mercer et al., 1991; Mercer and Crapo, 1993); units with the shorter paths are expected to have the greater damage. This could have significant implications for regional or localized damage to lung tissue. Whereas the average lung dose might be at a level that would be considered insignificant, local regions of the lung may receive significantly higher than average doses and therefore be at greater risk for chronic effects.

Theoretical models also have been applied to make predictions about delivered doses from exposure scenarios that are not necessarily achievable experimentally. Overton and

Graham (1989) and Miller and Overton (1989) have scaled the human lung dimensions to account for age variations. They predicted that LRT uptake efficiency is not sensitive to age at resting ventilation, but is age dependent when exercise conditions are invoked. The total quantity of O_3 absorbed per minute is predicted to increase with age during heavy work or exercise.

8.6.2 Species Homology and Sensitivity

Examining functional parameters measured analogously in humans and various animal species discloses remarkable similarity in acute O_3-induced effects. The tachypneic response to this oxidant is clearly concentration-dependent in both humans and animals and shows parallel exacerbation when hyperventilation (e.g., exercise or CO_2) is superimposed. Indeed, rodents appear to be slightly more responsive than humans in this regard. What is not known is whether this is evidence of pulmonary irritant sensitivity, perhaps as a prelude to toxicity, or whether tachypnea is a defensive posture taken by the respiratory system to minimize distal lung O_3 deposition. Airway or lung resistance in humans is not affected appreciably by acute exposure to O_3, except under conditions of heavy exercise; animals appear to need high-level exposures or special preparations that bypass nasal scrubbing. Dynamic lung compliance, on the other hand, tends to decrease across species. However, the evidence in both animals and humans is not as strong as one might expect, given the distal lung deposition of this poorly soluble oxidant.

Ozone-induced spirometric changes, the hallmark of response in humans, also occur in exposed rats, although the relative responsiveness of these alterations in the rodent appears to be about half that of the human. It is unclear, however, the degree to which anesthesia (rat) and the comparability of hyperventilation induced by CO_2 (rat) or exercise (human) may influence this difference in responsiveness. Collectively, the acute functional response of laboratory animals to O_3 appears quite homologous to that of the human. Likewise, the studies of BAL constituents indicate that the influx of inflammatory cells and protein from the serum is influenced by species, but perhaps to a less extent than by ventilation and antioxidant status, because adjustment for these factors can modulate responses to approximate animal responses to those of humans. Unfortunately, these influential factors are rarely measured and, even less often, controlled.

When humans are exposed repeatedly for several consecutive days, lung function decrements subside, and normal spirometric parameters are regained. This phenomenon of functional attenuation also has been demonstrated in rats, not only in terms of spirometry, but also in terms of the classic tachypneic ventilatory response. Full or partial attenuation of the BAL parameters also appears to occur in both rats and humans, but exposure scenario appears to play a role; other cellular changes in animals do not attenuate. Existing epidemiologic studies provide only suggestive evidence that persistent or progressive deterioration in lung function is associated with long-term oxidant-pollutant exposure. These long-term effects are thought to be expressed in the form of maximum airflow or spirometric abnormalities, but the foundation for this conclusion remains weak and hypothetical. Animal study data, although suggesting that O_3 has effects on lung function at near-ambient levels, present a variable picture of response that may or may not relate to technical conditions of exposure or some other, yet undiscovered variable of response. Thus, a cogent interpretation of the animal findings as definitive evidence of chronic deterioration of lung function would be difficult at this time. However, the subtle functional defects apparent after 12 to 18 mo of exposure and

the detailed morphometric assessments of the O_3-induced lesions do appear consistent with the modicum of studies focusing on long-term effects in human populations. Based on the apparent homology of these responses between humans and laboratory animals, animal studies provide a means to more directly assess such chronic health concerns.

8.6.3 Quantitative Extrapolation

The agreement between theoretical models of O_3 uptake and experimental determinations of O_3 uptake efficiency now provide a basis on which responses may be examined as a function of delivered O_3 dose instead of O_3 exposure concentration. By examining responses as a function of delivered dose, the goal of quantitative extrapolation between species can be approached.

The use of delivered dose to investigate responses has been examined in two contexts: (1) intraspecies comparisons and (2) interspecies comparisons. With respect to intraspecies comparisons, Miller et al. (1988) assumed that the relevant dose mediating the human pulmonary function response was the pulmonary tissue dose. They then utilized the breathing patterns, exposure concentrations, and pulmonary function responses from the human studies of McDonnell et al. (1983) to predict the dose-response. They found that there was general agreement between the shapes of the concentration-response curves and the dose-response curves and that differences could be accounted for by the translation between exposure concentration and O_3 dose. In another example dealing with intraspecies comparisons, Miller and Conolly (1995) compared the distribution of predicted O_3 tissue dose to a ventilatory unit in a rat as a function of distance from the BADJ, with the distribution of alveolar wall thickening as a function of the same distance measure. Miller and Conolly (1995) found remarkable consistency between the predicted dose distribution and the response distribution (i.e., as predicted delivered dose declined, response declined).

In an attempt to make an interspecies comparison of dose and response, existing or modified models of Miller et al. (1985), Miller et al. (1988), and Overton et al. (1987) were used to predict doses among species for two different types of responses. In the first case, the tachypneic response to O_3 as a function of dose was analyzed. The maximum ratio of O_3-altered f to control f was plotted as a function of the average centriacinar dose over the period from the beginning of exposure to the point of maximum f ratio. Rat and human data were used for this comparison. It was found that, at comparable O_3 doses, the responses of rats greatly exceeded that of humans and were initiated at lower doses. By examining the dose response instead of the concentration response, the difference in tachypneic response between rats and humans is magnified. In another example, an analysis similar to Miller et al. (1988) was performed to examine recovered BAL protein as a function of O_3 dose to the pulmonary region. The species considered were the rat, guinea pig, rabbit, and human. In all cases, the BAL protein response followed a log-linear relationship, suggesting a consistency of response across species. Yet the data from different species tended to cluster together, suggesting species-specific sensitivity factors.

An attempt was made to address quantitatively the question of potential chronic alteration of the lungs of people exposed to O_3 by integrating dosimetry model predictions and biological effects observed in laboratory animals. In the long-term exposure studies selected for analysis, importance was placed on the relevance of exposure concentrations, the site of specificity of the morphometric analysis, and the consistency of analysis within species. Two rat (F344) studies were selected that represented near-lifetime exposures to O_3 over a range of

concentrations and scenarios (Chang et al., 1992, 1995). Two monkey studies were also considered: (1) bonnet monkeys exposed for 90 days (Harkema et al., 1993) and (2) fascicularis monkeys exposed daily for 18 mo or daily every other month for 18 mo (Tyler et al., 1988). The biological effect chosen for extrapolation was the increased thickness of the acellular and total interstitial volumes in the PAR region of the lung; measurements were made in all four of these investigations. The quantity of O_3 predicted to be absorbed per square centimeter of PAR surface area was chosen as the dose.

Generally, the information needed to carry out the dosimetry predictions was not provided by the studies. This required assumptions such as the scaling of ventilation parameters, volumes, and surface areas from one species or strain to another. The assumption that had the greatest impact on the modeling results dealt with the pulmonary region mass transfer coefficient. The value used for this parameter has very little experimental justification and was chosen to be approximately the same for all species (i.e., the values calculated by Miller et al. [1985] for humans and by Overton et al. [1987] for rats). If the value of this coefficient is in fact approximately the same for all the species, the extrapolation of effects is not expected to be affected by the value itself. For the human simulations, a 9-year-old child and an adult outdoor supervisor living in New York City were considered. The activity and exposure patterns for these hypothetical people were generated by an exposure model (Johnson, 1994) for April through October 1991. The laboratory animal dose-response curves showed an apparent linear relationship within species with relatively high correlation coefficients, from 0.80 to 0.98 depending on species and effect. Assuming the relationships depicted in Figure 8-18, the predicted dose for the hypothetical humans indicated a seasonal response for the child of a 20 to 75% increase in PAR tissue thickness and, for the adult, a 15 to 70% increase, depending on the laboratory species used for the prediction (the higher range corresponds to the monkey). For the monkeys, there seemed to be little reversal with postexposure to air, which was consistent with the cumulative dose hypothesis. Although the reader should note the number of assumptions that underlie these predictions, this exercise, nevertheless, suggests that long-term O_3 exposure could impart a chronic effect in humans.

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